
FPL

CONTRAINDICATIONS

There is no known specific contraindication for NOVANTRONE. Accidental overdoses have been reported. Four patients receiving 140 - 180 mg/M² as a single bolus injection died as a result of severe leukopenia with infection. Hematologic support and antimicrobial therapy may be required during prolonged periods of medullary hypoplasia.

Although patients with severe renal failure have not been studied, NOVANTRONE is extensively tissue bound and it is unlikely that the therapeutic effect or toxicity would be mitigated by peritoneal or hemodialysis.

DOSAGE AND ADMINISTRATION (See WARNINGS)

NOVANTRONE SOLUTION MUST BE DILUTED PRIOR TO USE.

Combination Initial Therapy for ANLL in Adults. For induction, the recommended dosage is 12 mg/M² of NOVANTRONE daily on Days 1-3 given as an intravenous infusion, and 100 mg/M² of cytosine arabinoside for seven days given as a continuous 24 hour infusion on Days 1-7.

Most complete remissions will occur following the initial course of induction therapy. In the event of an incomplete antileukemic response, a second induction course may be given. NOVANTRONE should be given for two days and cytosine arabinoside for five days using the same daily dosage levels.

If severe or life-threatening nonhematologic toxicity is observed during the first induction course, the second induction course should be withheld until toxicity clears.

Consolidation therapy which was used in two large randomized multicenter trials consisted of NOVANTRONE 12 mg/M² given by intravenous infusion daily for days 1 and 2, and cytosine arabinoside, 100 mg/M² for 5 days given as a continuous 24 hour infusion on days 1-5. The first course was given approximately 6 weeks after the final induction course, the second was generally administered 4 weeks after the first. Severe myelosuppression occurred. (See CLINICAL PHARMACOLOGY Section)

NOVANTRONE solution should be diluted to at least 50 mL with either 0.9% Sodium Chloride Injection (USP) or 5% Dextrose Injection (USP). This solution should be introduced slowly into the tubing as a freely running intravenous infusion of 0.9% Sodium Chloride Injection (USP) or 5% Dextrose Injection (USP) over a period of not less than 3 minutes. Unused infusion solutions should be discarded in an appropriate fashion. If extravasation occurs, the administration should be stopped immediately and restarted in another vein. The nonvesicant properties of NOVANTRONE eliminate the possibility of severe local reactions following extravasation. However, care should be taken to avoid extravasation at the infusion site and to avoid contact of NOVANTRONE with the skin, mucous membranes or eyes.

Skin accidentally exposed to NOVANTRONE should be rinsed copiously with warm water and if the eyes are involved, standard irrigation techniques should be used immediately. The use of goggles, gloves, and protective gowns is recommended during preparation and administration of the drug. Spills on equipment and environmental surfaces may be cleaned using an aqueous solution of calcium hypochlorite (5.5 parts calcium hypochlorite in 13 parts by weight of water for each 1 part of NOVANTRONE). Absorb the solution with gauze or towels and dispose of these in a safe manner. Appropriate safety equipment such as goggles and gloves should be worn while working with calcium hypochlorite.

NOVANTRONE should not be mixed in the same infusion as heparin since a precipitate may form. Because specific compatibility data are not available, it is recommended that NOVANTRONE not be mixed in the same infusion with other drugs.

Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published.[1-6] There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate.

REFERENCES

1. Recommendations for the Safe Handling of Parenteral Antineoplastic Drugs. NIH Publication No 83-2621. For sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402
2. ASHP Council Report. Guidelines for Handling Parenteral Antineoplastics. JAMA, March 15, 1985
3. National Study Commission on Cytotoxic Exposure - Recommendations for Handling Cytotoxic Agents. Available from Laura P. Jeffrey Sc. D., Director of Pharmacy Services, Rhode Island Hospital, 593 Eddy Street, Providence, Rhode Island 02902
4. Clinical Oncological Society of Australia: Guidelines and recommendations for safe handling of antineoplastic agents. Med J Australia 1: 426-428 1983
5. Jones R.B., et al. Safe handling of chemotherapeutic agents: A report from the Mount Sinai Medical Center. Ca - A Cancer Journal for Clinicians Sept/Oct. 258-263 1983
6. American Society of Hospital Pharmacists technical assistance bulletin on handling cytotoxic drugs in hospitals. Am J Hosp Pharm 42: 131-137 1985

NOVANTRONE may be further diluted into Dextrose 5% in Water, Normal Saline or Dextrose 5% with Normal Saline and used immediately. DO NOT FREEZE.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

HOW SUPPLIED

NOVANTRONE® mitoxantrone hydrochloride concentrate for injection is a sterile aqueous solution of a concentration equivalent to 2 mg mitoxantrone free base per mL and is supplied in vials for single dose use as follows:

NDC 0005-9393-34 - 10 mL/vial (20 mg)
NDC 0005-9393-72 - 12.5 mL/vial (25 mg)
NDC 0005-9393-36 - 15 mL/vial (30 mg)

NOVANTRONE is stable for two years from time of manufacture. NOVANTRONE should be stored at Controlled Room Temperature 15-30°C (59-86°F). DO NOT FREEZE.

Manufactured by
LEDERLE LABORATORIES DIVISION
Cyanamid of Great Britain Ltd.
Jesport, Hampshire, England
Distributed by
LEDERLE LABORATORIES DIVISION
American Cyanamid Company,
Pearl River, NY 10965

REV 12/87

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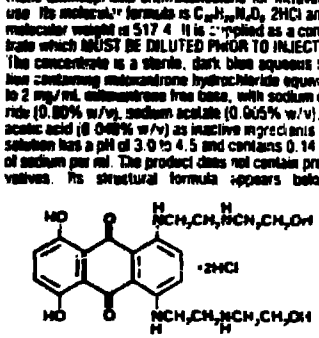
NOVANTRONE®
Mitoxantrone Hydrochloride
Concentrate For Injection

DESCRIPTION

NOVANTRONE® mitoxantrone hydrochloride is a synthetic antineoplastic anthracenedione for intravenous use. Its molecular formula is $C_{22}H_{28}N_4O_6 \cdot 2HCl$ and its molecular weight is 517.4. It is supplied as a concentrate which MUST BE DILUTED PRIOR TO INJECTION. The concentrate is a sterile, dark blue aqueous solution containing mitoxantrone hydrochloride equivalent to 2 mg/mL mitoxantrone free base, with sodium chloride (0.80% w/v), sodium acetate (0.065% w/v), and acetic acid (0.048% w/v) as inactive ingredients. The solution has a pH of 3.0 to 4.5 and contains 0.14 mEq of sodium per mL. The product does not contain preservatives. Its structural formula appears below.



1,4-Dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione dihydrochloride

CLINICAL PHARMACOLOGY

Although its mechanism of action is not fully elucidated, NOVANTRONE is a DNA-reactive agent. It has a cytocidal effect on both proliferating and nonproliferating cultured human cells, suggesting lack of cell cycle phase specificity. Pharmacokinetic studies have not been performed in humans receiving multiple daily doses. Pharmacokinetic studies in adult patients following a single intravenous administration of NOVANTRONE have demonstrated multi-exponential plasma clearance. Distribution to tissues is rapid and extensive. Distribution to the brain, spinal cord, eye, and spinal fluid in the monkey is low. The apparent steady state volume of distribution exceeds 1000L/M². Elimination of drug is slow with an apparent mean terminal plasma half-life of 5.8 days (range 2.3-13.5). The half-life in tissues may be longer. Multiple intravenous doses in dogs daily for five days resulted in significant accumulation in plasma and tissue. The extent of accumulation was four fold.

NOVANTRONE is 78% bound to plasma proteins in the observed concentration range of 26-456 ng/mL. This binding is independent of concentration and was not affected by the presence of diphenylhydantoin, doxorubicin, methotrexate, prednisone, prednisolone, heparin, or acetylsalicylic acid.

NOVANTRONE is excreted via the renal and hepatobiliary systems. Renal excretion is limited, only 5% - 11% of the dose is recovered in the urine within five days after drug administration. Of the material recovered in the urine, 65% is unchanged drug, the remaining 35% is comprised primarily of two inactive metabolites and their glucuronide conjugates. The metabolites are mono- and dicarboxylic acid derivatives. Hepatobiliary elimination of drug appears to be of greater significance with as much as 25% of the dose recovered in the feces within five days of intravenous dosing. No significant differences in the pharmacokinetics of NOVANTRONE was observed in 7 patients with moderately impaired liver function (serum bilirubin 1.3-3.4 mg/dL) as compared with 15 patients without hepatic dysfunction. Results of pharmacokinetic studies on 4 patients with severe hepatic dysfunction (bilirubin greater than 3.4 mg/dL) suggest that these patients have a lower total body clearance and a larger Area under Curve than do the patients at a comparable NOVANTRONE dose.

In two large randomized multicenter trials remission induction therapy for ANLL with NOVANTRONE 12 mg/M² daily for three days as a 10-minute intravenous infusion and cytosine arabinoside 100 mg/M² for seven days given as a continuous 24 hour infusion was compared with daunorubicin 45 mg/M² daily by intravenous infusion for three days plus the same dose and schedule of cytosine arabinoside used with NOVANTRONE. Patients who had an incomplete antileukemic response received a second induction course in which NOVANTRONE or daunorubicin was given for two days and cytosine arabinoside for five days using the same daily dosage schedule. Response rates and median survival information for both the U.S. and international multicenter trials are given in the following table:

Trial	% Complete Response(CR)		Median Time to CR (days)		Median Survival (days)	
	NOV	DAUN	NOV	DAUN	NOV	DAUN
U.S.	63 (62/98)	53 (54/102)	35	42	312	237
Foreign	50 (56/112)	51 (62/123)	36	42	192	230

NOV = NOVANTRONE® + Cytosine arabinoside
DAUN = Daunorubicin + Cytosine arabinoside

In these studies, two consolidation courses were administered to complete responders on each arm. Consolidation therapy consisted of the same drug and daily dosage used for remission induction, but only 5 days of cytosine arabinoside and 2 days of NOVANTRONE or daunorubicin were given. The first consolidation course was administered 8 weeks after the start of the final induction course if the patient achieved a com-

plete remission. The second consolidation course was generally administered 4 weeks later. Full hematologic recovery was necessary for patients to receive consolidation therapy. For the U.S. trial, median granulocyte nadirs for patients receiving NOVANTRONE + cytosine arabinoside for consolidation courses 1 and 2 were 10/mm³ for both courses, and for those patients receiving daunorubicin + cytosine arabinoside were 170/mm³ and 200/mm³, respectively. Median platelet nadirs for patients who received NOVANTRONE + cytosine arabinoside for consolidation courses 1 and 2 were 17,000/mm³ and 14,000/mm³, respectively, and were 33,000/mm³ and 22,000/mm³ in courses 1 and 2 for those patients who received daunorubicin + cytosine arabinoside. The benefit of consolidation therapy in AMLL patients who achieve a complete remission remains controversial. However, in the only well controlled prospective, randomized multicenter trials with NOVANTRONE in AMLL, consolidation therapy was given to all patients who achieved a complete remission. During consolidation in the U.S. study, five myelosuppression related deaths occurred on the NOVANTRONE arm and one on the daunorubicin arm. However, in the foreign study there were eight deaths on the NOVANTRONE arm during consolidation which were related to the myelosuppression and none on the daunorubicin arm where less myelosuppression occurred.

INDICATIONS AND USAGE

NOVANTRONE in combination with other approved drug(s) is indicated in the initial therapy of acute nonlymphocytic leukemia (AMLL) in adults. This category includes myelogenous, promyelocytic, monocytic, and erythroid acute leukemias.

CONTRAINDICATIONS

NOVANTRONE is contraindicated in patients who have demonstrated prior hypersensitivity to it.

WARNINGS

WHEN NOVANTRONE IS USED IN DOSES INDICATED FOR THE TREATMENT OF LEUKEMIA, SEVERE MYELOSUPPRESSION WILL OCCUR. THEREFORE, IT IS RECOMMENDED THAT NOVANTRONE BE ADMINISTERED ONLY BY PHYSICIANS EXPERIENCED IN THE CHEMOTHERAPY OF THIS DISEASE. LABORATORY AND SUPPORTIVE SERVICES MUST BE AVAILABLE FOR HEMATOLOGIC AND CHEMISTRY MONITORING AND ADJUNCTIVE THERAPIES INCLUDING ANTIBIOTICS. BLOOD AND BLOOD PRODUCTS MUST BE AVAILABLE TO SUPPORT PATIENTS DURING THE EXPECTED PERIOD OF MEDULLARY HYPOPLASIA AND SEVERE MYELOSUPPRESSION. PARTICULAR CARE SHOULD BE GIVEN TO ASSURING FULL HEMATOLOGIC RECOVERY BEFORE UNDERTAKING CONSOLIDATION THERAPY (IF THIS TREATMENT IS USED) AND PATIENTS SHOULD BE MONITORED CLOSELY DURING THIS PHASE.

Patients with pre-existing myelosuppression as the result of prior drug therapy should not receive NOVANTRONE unless it is felt that the possible benefit from such treatment warrants the risk of further medullary suppression. Because of the possible danger of cardiac effects in patients previously treated with daunorubicin or doxorubicin, the benefit-to-risk ratio of NOVANTRONE therapy in such patients should be determined before initiating therapy.

The safety of NOVANTRONE in patients with hepatic insufficiency is not established. (See CLINICAL PHARMACOLOGY.)

Cardiac Effects

General - Functional cardiac changes including congestive heart failure and decreases in left ventricular ejection fraction (LVEF) occur with NOVANTRONE. Cardiac toxicity may be more common in patients with prior treatment with anthracyclines, prior mediastinal radiotherapy, or with pre-existing cardiovascular disease. Such patients should have regular cardiac monitoring of LVEF from the initiation of therapy. In investigational trials of intermittent single doses in other tumor types, patients who received up to the cumulative dose of 140 mg/M² had a cumulative 7.8% probability of clinical congestive heart failure. The overall cumulative probability rate of moderate or serious decreases in LVEF at this dose was 13% in comparative trials.

Leukemia - Acute CHF may occasionally occur in patients treated with NOVANTRONE for AMLL. In first line comparative trials of NOVANTRONE + cytosine arabinoside vs daunorubicin + cytosine arabinoside in adult patients with previously untreated AMLL, therapy was associated with congestive heart failure in 6.5% of patients on each arm. A causal relationship between drug therapy and cardiac effects is difficult to establish in this setting since myocardial function is frequently depressed by the anemia, fever and infection and hemorrhage which often accompany the underlying disease.

Pregnancy Category D - NOVANTRONE may cause fetal harm when administered to a pregnant woman. In treated rats, low fetal birth weight and retarded development of the fetal kidney were seen in greater frequency. In rabbits, an increased incidence of premature delivery was observed. NOVANTRONE was not teratogenic in rabbits. There are no adequate and well-controlled studies in pregnant women. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant.

Safety for use by routes other than intravenous administration has not been established.

PRECAUTIONS

General: Therapy with NOVANTRONE should be accompanied by close and frequent monitoring of hematologic and chemical laboratory parameters, as well as frequent patient observation.

Hyperuricemia may occur as a result of rapid lysis of tumor cells by NOVANTRONE. Serum uric acid levels should be monitored and hyperuricemic therapy instituted prior to the initiation of antileukemic therapy.

Systemic infections should be treated concomitantly with or just prior to commencing therapy with NOVANTRONE.

Information for Patients: NOVANTRONE may impart a blue-green color to the urine for 24 hours after administration and patients should be advised to expect this during therapy. Bluish discoloration of the sclera may also occur. Patients should be advised of the signs and symptoms of myelosuppression.

Laboratory Tests: *Serial complete blood counts and liver function tests are necessary for appropriate dose adjustments.* (See DOSAGE AND ADMINISTRATION section.)

Carcinogenesis, Mutagenesis: NOVANTRONE can result in chromosomal aberrations in animals and it is mutagenic in bacterial systems. NOVANTRONE caused DNA damage and sister chromatid exchanges in vitro.

Pregnancy Category D (See WARNINGS section.)

Nursing Mothers: It is not known whether NOVANTRONE is excreted in human milk. Because of the potential for serious adverse reactions in infants from NOVANTRONE, breast feeding should be discontinued before starting treatment.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

NOVANTRONE has been studied in approximately 600 patients with acute nonlymphocytic leukemia. The table below represents the adverse reaction experience in the large U.S. comparative study of mitoxantrone plus cytosine arabinoside vs daunorubicin plus cytosine arabinoside. Experience in the large foreign study was similar. A much wider experience in a variety of other tumor types revealed no additional important reactions other than cardiomyopathy (See WARNINGS). It should be appreciated that the listed adverse reaction categories include overlapping clinical symptoms related to the same condition, e.g. dyspnea, cough and pneumonia. In addition, the listed adverse reactions may not all necessarily be attributed to chemotherapy as it is often impossible to distinguish effects of the drug and effects of the underlying disease. It is clear, however, that the combination of NOVANTRONE plus cytosine arabinoside was responsible for nausea and vomiting, alopecia, mucositis, stomatitis, and myelosuppression.

The following table summarizes adverse reactions occurring in patients treated with NOVANTRONE + cytosine arabinoside in comparison with those who received daunorubicin + cytosine arabinoside for therapy of AMLL in a large multicenter randomized prospective U.S. trial. Adverse reactions are presented as major categories and selected examples of clinically significant subcategories.

	ALL INDUCTION (percentage of pts entering induction)		ALL CONSOLIDATION (percentage of pts entering consolidation)	
	NOV N = 102	DAUN N = 102	NOV N = 55	DAUN N = 49
Cardiovascular	26	28	11	24
CHF	5	6	0	0
Arrhythmia	3	3	4	4
Bleeding	37	41	20	6
GI	16	12	2	2
petechiae/ecchymoses	7	9	11	2
Gastrointestinal	88	85	58	51
Nausea/Vomiting	72	67	31	31
Diarrhea	47	47	18	8
Abdominal Pain	15	9	9	4
Mucositis/Stomatitis	29	33	18	8
Hepatic	10	11	14	2
Jaundice	3	8	7	0
Infections	66	73	60	43
UTI	7	7	7	2
pneumonia	9	7	5	0
sepsis	34	36	31	18
fungal infections	15	13	9	6
Renal Failure	6	6	0	2
Fever	73	71	24	18
Alopecia	37	40	22	16
Pulmonary	43	43	24	14
Cough	13	9	9	2
Dyspnea	18	20	6	0
CNS	30	30	34	35
Seizures	4	4	2	6
Headache	10	9	13	3
Eye	7	6	2	4
Conjunctivitis	5	3	0	0

Allergic Reaction Hypotension, urticaria, dyspnea and rashes have been reported occasionally.

Cutaneous Phlebitis has been reported infrequently at the site of infusion. There have been rare reports of tissue necrosis following extravasation.

Hematologic Myelosuppression is rapid in onset and is consistent with the requirement to produce significant marrow hypoplasia in order to achieve a response. The incidences of infection and bleeding seen in the U.S. trial are consistent with those reported for other standard induction regimens.

Gastrointestinal Nausea and vomiting occurred acutely in most patients, but were generally mild to moderate and could be controlled through the use of antiemetics. Stomatitis/mucositis occurs within one week of therapy.

Cardiovascular Congestive heart failure, tachycardia, EKG changes including arrhythmias, chest pain and asymptomatic decreases in left ventricular ejection fraction have occurred (see WARNINGS).

22055

NDA 19-297

Lederle Laboratories
A Division of American Cyanamid Company
Pearl River, New York 10965

Attention: Dennis J. Foley, Ph.D.
Director
Regulatory Liaison

APR 5 1988

Dear Dr. Foley:

We acknowledge the receipt of final printed labeling (FPL) dated January 19, 1988 for your approved new drug application for NOVANTRONE (mitoxantrone hydrochloride) Concentrate for Injection.

We have reviewed the FPL that you have submitted in accordance with our approval letter dated December 23, 1987, and we find it acceptable.

Sincerely yours,

John P. Palmer, M.D.
Director
Division of Oncology and
Radiopharmaceutical Drug Products
Office of Drug Research and Review
Center for Drug Evaluation and Research

cc.
Original NDA 19-297
HFN-150/Div. File
HFN-80
HFN-83
HFN-85(3)
HFN-150/GBurke
HFN-730(1)
HFN-232(1)
HFN-150/AMSindelar/1-27-88
R/D Init by: G.Burke/1-28-88
J.R.Johnson/1-28-88
E.Tolgyesi/1-28-88
R.H.Wood/2-1-88
D.Richman/2-2-88
R.G.Scully/3-16-88
K.Y.Lo/HFN-226
R.Stein/HFN-713
F/T:dlb/3-30-88 Wang #2356E

FPL ACCEPTABLE Ltr; Retain

STAT

REV

Statistical Review and Evaluation

Date: DEC 14 ...

NDA#: 19-297/ Drug Class: 3C

Applicant: Lederle Laboratories

Name of Drug: Novantrone (mitoxantrone)

Documents Reviewed: Volumes 14.1-14.3, 15.1-15.2 dated respectively 10/02/87 and 10/22/87 by the applicant.

The reviewing medical officer for this submission is Gregory Burke, M.D., HFN-150, who is in general agreement with this review.

1. Background

Novantrone in combination with Ara-C is proposed for the first line treatment of adult acute non-lymphocytic leukemia (ANLL). Only two multicenter studies submitted for this indication are considered by the applicant to be prospective randomized, controlled phase III clinical trials. These are protocols 3-74 and 3-603.

2. Study Characteristics

Protocol 3-74 was designed to be a randomized, controlled, open label multicenter study of previously untreated patients with ANLL. Patients in the United States were treated with Novantrone and Ara-C or daunorubicin and Ara-C. Patient allocation was stratified on age (<60 , ≥60) years. This leads to 50 patients in each cell of the four treatment by stratum combinations and a total of 200 patients in the study. The actual trial began in the first quarter of 1984 and was scheduled to last for 2-3 years.

Protocol 3-603 was to be a randomized, controlled, open label multicenter study of previously untreated patients having ANLL. Patients outside the United States were treated with Novantrone and Ara-C or daunorubicin and Ara-C. A total of 200 patients were to be included in the trial. The actual trial began in the first quarter of 1985 and was scheduled to last for 2-3 years.

The basic characteristics of the completed studies are summarized below.

Protocol	Treat-ment	No. of Invest.	No. of Patients	No. of Deaths(%)	Random-ized	Blinding	Therapy
3-74	N+A [1]	27	98	61 (62%)	yes	Open	1st line
	d+A		102	66 (65%)			
3-603	N+A	18	116 [2]	74 (66%)	yes [3]	Open	1st line
	d+A		123	70 (57%)			

[1] N+A represents the treatment combination of Novantrone and Ara C
d+a represents the treatment combination of daunorubicin and Ara C

[2] In protocol 3-74, the applicant recruited the number of patients specified by protocol. In protocol 3-603, 20% more patients (39) than specified by protocol were recruited.

[3] In protocol 3-603, treatment allocation errors occurred. In Hong Kong, 9/15 patients were not allocated treatment as randomized. In Taiwan, 1/16 patients was not allocated treatment as randomized.

The applicant treated complete remission (CR) rate, CR duration, time to treatment failure (TTF), and survival time as primary efficacy variables.

3. Applicant's Analyses

The applicant applied the same general statistical methods in studies 3-74 and 3-603. The applicant analyzed only "evaluable" patients rather than doing "intent to treat" analyses. This means that only patients who were correctly diagnosed and received treatment were analyzed for efficacy. In the opinion of Dr. Burke, HFN-150, additional primary efficacy analyses excluding only incorrectly diagnosed patients should be performed. Primary efficacy variables were Complete Remission (CR) rates and Survival. Secondary efficacy variables were CR Duration, Time to CR, and Time to Treatment Failure (TTF). In the protocol, it was indicated that one-sided statistical procedures were to be used. In the actual submission, two-sided procedures were used instead.

Confidence intervals for CR rates and CR rate differences were computed overall and within subgroups using the standard normal approximation to the binomial distribution. P-value comparisons of CR rates were based on Fisher's Exact Test. Survival curves, median survival times, and confidence intervals for median survival time were based on Kaplan-Meier estimation, Miller's interpolation of the Kaplan-Meier estimated survival

function, and the application of Greenwood's formula to define a 95% confidence region for the survival function. These survival estimators were applied overall and within patient subgroups. Statistical differences in overall survival curves were tested using the logrank and Gehan tests. Stratified survival data were compared across treatment groups using a stratified logrank test. The hazard ratio was estimated assuming simple exponential survival within each treatment group. Confidence intervals on the hazard ratio were based on log-normal approximations of the hazard rates.

In study 3-603, unplanned subgroup analyses were performed by "geographic region". The applicant called these the European, the Latin American, and the pacific regions. The applicant justified these subgroup analyses because " Due to the potential differences in patient population and supportive care in the 3 regions, overall and regional analyses were conducted." (Vol.15.1, page 190).

4. Applicant's Claimed Results

a. Study 3-74

The applicant's conclusion section states (Vol. 14.1, pages 118-122):
"The substitution of Novantrone for Cerubidine (daunorubicin) in combination with Ara-C in the first line therapy of ANLL resulted in comparable efficacy and safety in this prospective randomized trial. There were no statistically significant differences in the complete response rates, CR duration, TTF, and survival. Both regimens are highly active in the treatment of previously untreated patients with ANLL. .... The adverse experience profiles in patients treated with either of the two regimens were comparable in incidence and in severity."

The applicant then proceeded to discuss observed CR rates, median survival times, and adverse event rates. Except for restating the statistically non-significant survival difference, the applicant made no use of probability based arguments in the conclusion section I just quoted from.

b. Study 3-603

The applicant concluded (Vol. 15.1, pages 277-281):
"This multicentre, prospectively randomized, comparative study demonstrated that substituting Novantrone for Cerubidine, both in combination with Ara-C, in the first line of ANLL resulted in comparable efficacy and safety. There were no statistically significant differences in response rate, response duration, TTF, or survival. ...."

As in study 3-74, the applicant made little use of supporting probability arguments in the conclusions section of study 3-603.

5. Reviewer's Comments

In the opinion of Dr. Burke, HFN-150, additional primary efficacy analyses excluding only incorrectly diagnosed patients should be performed by the applicant. Currently lacking these analyses, the next step is to examine the results of the applicant's basic/standard analyses of primary efficacy variables.

(A) The applicant's statistical analysis of study 3-603 by geographic region appear to be a data driven attempt to discard unfavorable patient survival and CR results for Novantrone. The initial motivation for such a breakdown by region is the misallocation of some patients to treatment in Taiwan and Hong Kong. This is the point of departure for the applicant to suggest that the supportive care in the pacific region, which included Taiwan and Hong Kong, was not as good as elsewhere. The applicant implied that overall results were biased against Novantrone as a result of this poorer supportive care. This is then used as a reason to drop the entire pacific region from the complete analysis of study 3-603. In my opinion, only after one looks at the actual data can reasons why results were biased against Novantrone be constructed.

The data driven appearance of the applicant's analyses is further supported by the fact that there is no provision in protocol 3-603 for any analytic or other need to consider geographic differences in supportive care. When Europe is constructed by the applicant to be made up of Sweden, Israel, and South Africa, the geographic differences in support care between Europe, Latin America and the pacific seem artificial.

(B) In study 3-74, the applicant found that the lower 2-sided 95% confidence bound computed for the mortality hazard ratio was 0.80. This means that the survival time of daunorubicin patients could be 25% (= 100 x [(1/0.80)-1]) longer than for Novantrone patients despite the fact that the observed survival time of Novantrone patients was longer than that for daunorubicin patients (Vol. 14.1, page 136). The applicant has rightfully displayed the survival results for each age stratum and has rightfully avoided any subgroup interpretation of these statistical results. I see no reason to consider the hazard ratios for these two strata as estimating different values. Consequently, under exponential survival conditions, the applicant's lower bound of 25% less survival on Novantrone appears acceptable to this reviewer.

The applicant also found the observed CR rate for Novantrone was 10% higher than for daunorubicin, and the corresponding 95% confidence interval ranged from a 4% remission advantage favoring daunorubicin to a 24% remission advantage favoring Novantrone (Vol. 14.1, page 121).

(C) In study 3-603, the applicant found that the lower 2-sided 95% confidence bound computed for the mortality hazard ratio was 0.60. This means that the survival time of daunorubicin patients could be 67% (= 100 x [(1/0.60)-1]) longer than for Novantrone patients (Vol. 15.1, page 341). The survival curve comparison p-value $\geq$ 0.15, which is not statistically significant, can not be interpreted in this study as showing survival comparability of the two treatment regimens. The applicant's interpretation of survival comparability of Novantrone + Ara-C and daunorubicin + Ara-C in study 3-603 (Vol. 15.1, page 277) which was based solely on statistical non-significance is inappropriate.

The applicant also found the observed CR rate for Novantrone was 1% lower than for daunorubicin, and the corresponding 95% confidence interval ranged from a 14% remission advantage favoring daunorubicin to a 12% remission advantage favoring Novantrone (Vol. 15.1, page 192).

(D) This reviewer finds the applicant's statistical methods, used to obtain the survival time and CR rate results stated in (B) and (C) above for studies 3-74 and 3-603, are satisfactory. In the opinion of Dr. Burke, HFN-150, the evidence in item (B) provides adequate clinical evidence in study 3-74 to show that Novantrone makes as much contribution to patient survival and CR rate as daunorubicin when used in conjunction with Ara-C. However, it is Dr. Burke's opinion that the evidence in item (C) may not show the comparable contribution of Novantrone and daunorubicin to Ara-C in terms of patient survival.

(E) In view of (D), how could the applicant conclude that the results of study 3-603 showed comparable effectiveness of Novantrone and daunorubicin when combined with Ara-C? The applicant, in fact, provided a variety of options. In the applicant's summary conclusions, the applicant's only probability based argument, i.e. based on p-values or confidence intervals, was that no statistically significant differences were found in CR rate, CR duration, TTF, or survival between the two treatment regimens (Vol. 15.1, page 277, paragraph 1). The role the applicant gave to lack of statistical significance is illustrated by the following paragraph in the study synopsis (Vol. 15.1, page 163) "It is <u>important</u> to note that none of the efficacy parameters for the <u>overall study</u> showed any statistically significant difference." This reviewer has added the underlining in this quotation for emphasis. I would like to make the following point.

Lack of a statistically significant treatment difference does not constitute statistical evidence of no treatment difference. In fact, lack of evidence of a treatment difference of any kind is just what it says it is, i.e. lack of evidence. What is being sought is positive evidence (i.e. primary evidence) that Novantrone in combination with Ara-C is comparable to daunorubicin, not the secondary lack-of-evidence arguments given by the applicant.

(F) In study 3-603, the applicant provided a second option for concluding that Novantrone with Ara-C was comparable to daunorubicin in effectiveness. In the applicant's words, "The hazard ratios [with 95% C.L.] calculated for each region separately are 1.27 [0.65, 2.48], 0.98 [0.61, 1.56] and 0.46 [0.23, 0.90] for Europe, Latin America and Pacific, respectively, (Table 10b) confirming this difference between the regions." (quote from Vol. 15.1, page 211). When the pacific region was omitted from the analysis of patient deaths, the daunorubicin/Novantrone hazard ratio lower bound became 0.72. This figure of 0.72 translates into an upper bound for the survival time of daunorubicin patients which is at most 39% longer than for Novantrone patients. This bound is clearly more acceptable than the applicant's original 67% survival deficit cited in item (C) of this section. I will now explain why, in this case, I consider it to be unacceptable to ignore the pacific region data.

** Independent evidence of effectiveness is what we are looking for. In this spirit, the results of study 3-74 should not prejudice the interpretation of study 3-603. Therefore, there is no prior substantial evidence that the treatment regimens being studied are equivalent. But, if one starts with the yet unsubstantiated belief that the two treatment regimens are equivalent (having a hazard ratio = 1), then the confidence intervals just given could indicate something wrong with the pacific region. This would be because the hazard ratio = 1 does not lie in the pacific region confidence interval [0.23, 0.90]. Unfortunately, removing the pacific region is based on the invalid assumption that the two treatment regimens are equivalent. Any "evidence" that drugs are equivalent which is based on the postulate that the drugs are equivalent is no "evidence" at all. It is rather an exercise in circular, self-fulfilling consistency.

** Simply looking at these 3 confidence intervals leads me to believe the data for the three geographic regions are consistent with a mortality hazard ratio between the values of 0.65 and 0.90. This range of hazard ratios indicates patients treated with daunorubicin plus Ara-C live longer than comparably treated Novantrone patients. Note that this interpretation of study 3-603, which does not make prior use of the results of study 3-74, is in fact consistent with study 3-74 as summarized in (B) above.

The following comment has no direct relevance to the review of the present submission for Novantrone in leukemia.

(G) The applicant has supplemented CR rate statistics with measures and comparisons of time to CR and CR duration. In my opinion, the applicant has introduced two new quantities which are statistically more relevant than CR rate alone. In fact, I believe these new quantities have the potential of making CR rate a secondary rather than a primary efficacy variable.

6. Comments which may be Conveyed to the Applicant

3(i) In study 3-74, the applicant's statistical methods for studying patient mortality and complete remission rates are acceptable. However, more than one third of the patients who can still be observed to die are still alive in each treatment group. It is not clear that study 3-74 will continue to show Novantrone in combination with Ara-C provides ANLL patients with comparable survival to daunorubicin when it has been virtually completed.

(ii) The statistical reviewer questions whether there is adequate statistical evidence in study 3-603 to conclude that Novantrone in combination with Ara-C provides first line ANLL patients with comparable complete remission rates and survival to daunorubicin with Ara-C. The lower bound on the two-sided 95% confidence interval for the mortality hazard ratio of all evaluable patients is 0.60. This implies that the daunorubicin combination could provide 67% longer patient survival time than the Novantrone combination. Furthermore, based on the applicant's 95% confidence intervals, the complete remission rate for patients in study 3-603 could be 14% worse for the Novantrone treated patients. Medical judgment is needed to decide whether differences this large can be considered as showing effectiveness comparable to that of patients treated with daunorubicin and Ara-C.

(iii) In study 3-603, the splitting of these clinical trial data into geographic regions is exploratory, and potentially data driven. The applicant has provided no statistical justification for the exclusion of patients in the pacific region on the basis of survival or complete remission rate. First, the original protocol defined no such geographic regions. This clearly does not substantiate the applicant's assertion that it was known beforehand that study 3-603 would have to be considered by region as well as overall.

Richard A. Stein, Ph.D.
Mathematical Statistician

This review with the Appendix has 14 pages.

Concur: Dr. Leung 12/11/87

Dr. Dubey 12-14-87

cc:
Orig. NDA 19-297
HFN-150
HFN-150/Dr. Burke
HFN-150/Dr. Johnson
HFN-344/Dr. Lisook
HFN-713/Dr. Dubey [File: DRU 1.3.2 NDA]
HFN-713/Dr. Stein
Chron.
R.A. Stein/x4594/SERB:ras:12/11/1987:NOV0002

APRVL

LTR

NDA 19-297

DEC 23 1987

Lederle Laboratories
A Division of American Cyanamid Company
Pearl River, New York 10965

Attention: Dennis J. Foley, Ph.D.
Director
Regulatory Liaison

Dear Dr. Foley:

Reference is made to your new drug application dated May 18, 1984 submitted pursuant to section 505(b) of the Federal Food, Drug, and Cosmetic Act for NOVANTRONE (Mitoxantrone Hydrochloride Concentrate for Injection).

We also acknowledge receipt of your additional communications dated March 12 and 19, May 15, September 21 and 30, October 22, December 10, 22 and 23, 1987.

We have completed the review of this application including the submitted draft labeling and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as initial therapy for acute nonlymphocytic leukemia (ANLL) in adults as recommended in the submitted draft labeling (package insert) as revised in the enclosure. In addition, the vial label and cartons must be revised as follows:

1. The vial and carton labels must be changed from NOVANTRONE Mitoxantrone Hydrochloride for Injection to NOVANTRONE (Mitoxantrone Hydrochloride Concentrate for Injection).

2. "AFTER DILUTION" must be emphasized (i.e., underline, block in yellow, enlarge or use different print) on the vial label and carton.

3. These revisions must be made immediately. However, your vial cartons for 20 mg (28,700), 25 mg (3,000) and 30 mg (3,000) of NOVANTRONE may be shipped with the provision that a prominent notice accompany each box of vials. This notice should, in bold print, clearly state that NOVANTRONE solution is a concentrate and must be diluted, and that revised labeling for the vial cartons will be implemented for further distribution of the drug.

Accordingly, the application, with the labeling revisions described above, is approved effective as of the date of this letter. Furthermore, it was agreed to on December 14, 1987 during a meeting with representatives of your staff, specifically, Drs. Kenneth Cartwright and Steven Saletan, and Dr. Gregory Burke and Ms. Alata Sindelar of this Division, that your firm will continue to follow the patients entered in the controlled clinical trials in ANLL and to submit yearly updates of the survival data as well as data regarding possible chronic toxicities.

Although the submitted data suggest activity for NOVANTRONE in relapsed ANLL, there are no adequate and well-controlled studies contained in the submission that demonstrate its safety and effectiveness for relapsed or refractory ANLL. Due to the heterogeneity of these patient populations, well-controlled studies are necessary to demonstrate effectiveness for these indications.

Moreover, neither do the mature data submitted for use of NOVANTRONE for the treatment of breast cancer provide adequate evidence that the drug will have the effect it purports or is represented to have under the conditions of use prescribed, recommended or suggested in its proposed labeling as required under 21 CFR 314.125(b)(5). Specifically, Study 4-32 (NOVANTRONE versus ADRIAMYCIN) does not provide evidence that treatment of patients with advanced breast cancer with NOVANTRONE will favorably affect their quality of life or survival. The low response rate (15%) does not imply efficacy in view of the sometimes severe toxicity. The design and outcome of Study 3-48 [CYTOXAN + NOVANTRONE + FLUOROURACIL (CNF) versus CYTOXAN + ADRIAMYCIN + FLUOROURACIL (CAF)] does not permit a conclusion that NOVANTRONE when used in combination with CYTOXAN and FLUOROURACIL is effective. The comparable survival to CAF alone does not provide evidence that NOVANTRONE is independently effective in this combination because the role of the substituted drug in enhancement of survival in this combination is not established. The inferior response rate of CNF taken with the lower bound of the 95% confidence limit of the difference in response rate likewise does not provide assurance that NOVANTRONE contributes to this effect in this combination. Therefore, we consider the indication not approvable for the use of NOVANTRONE as a second line treatment of breast cancer or in combination therapy for front line therapy.

The enclosed revisions in the draft package insert are terms of the NDA approval. Marketing the product before making the revisions, exactly as requested and previously agreed upon, in the product's final printed labeling (FPL) may render the product misbranded and an unapproved new drug.

Please submit twelve copies of the revised PPL when it is available. This submission should be designated for administrative purposes as "PPL Supplement" to the approved NDA 19-297. Approval of the supplement by FDA is not required before the labeling is used.

Should additional information relating to the safety and effectiveness of this drug product become available prior to our receipt of the PPL, further revision of that labeling may be required. Further recommendations for revisions in the labeling may be requested at the completion of the review by the Division of Biometrics of the carcinogenicity studies submitted March 12 and 19, 1987.

You may propose, at a later date, to include a section in the package insert claiming an appropriate storage time for the diluted solutions upon the submission of supportive chemical and microbiological data.

Please submit one market package of the drug when it is available.

In addition, please submit, in duplicate, the advertising copy which you intend to use in your proposed introductory promotional and/or advertising campaign. Please submit one copy to the Division of Oncology and Radiopharmaceutical Drug Products, and the second copy to the Division of Drug Advertising and Labeling, HFN-240, Room 10B-04, 5600 Fishers Lane, Rockville, Maryland 20857. Please submit all proposed materials in draft or mock-up form, not final print. Also, please do not use form FD-2253 for this submission; this form is for routine use, not proposed materials.

We remind you that you must comply with the requirements set forth under 21 CFR 314.80 and 314.81 for an approved NDA.

Sincerely yours,

Robert Temple, M.D.
Director
Office of Drug Research and Review
Center for Drug Evaluation and Research

cc.
Original NDA 19-297
HFT-150/Div. File
HFT-100/RTemple
HFN-80
HFN-83
HFN-150/GBurke
HFN-730
HFN-232
HFN-150/AMSindelar/12-11 & 23-87
S/D Init by: G.Burke/12-14 & 23-87
J.R.Johnson/12-14-87/G.Burke for J.J.12-23-87
E.Tolgyesi/12-15-87
R.H.Wood/12-15 & 23-87
D.Richman/12-14-87
K.Y.Lo/elected not to sign
J.P.Skelly/elected not to sign
R.Stein/12-15-87
S.Dubey/12-15-87
R.C.Scully/12-15-87
R.Jerussi/12-16-87
J.F.Palmer/12-16-87
F/T:ka:12-23-87
Wang #2292E

APPROVED NDA

JBalmer 12-23-87

12/23/87

BIO/DIS

REV

Mitoxantrone HCl (NOVANTRONE™)
(For injection)
NDA 19-297
Reviewer: Ko-Yu Lo, Ph.D.
Wang [redacted]
13-S
2-D
2-0

Lederle Laboratory
Pearle River, NY 10965

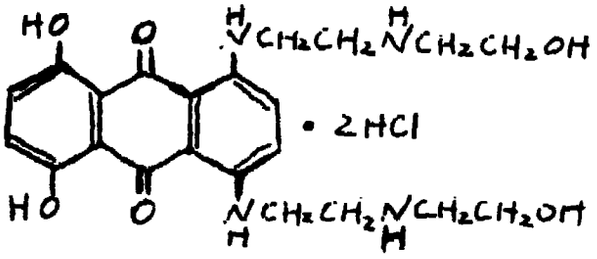
Submission Dated:
May 15, 1984
October 21, 1985

MAR 5 1986

Review of: (1) 5 clinical pharmacokinetic studies.
(2) 3 clinical pharmacokinetic studies from Literature.
(3) 1 metabolism study in human.
(4) 2 in vitro protein binding studies.
(5) 4 animal studies related to the drug's tissue distribution, accumulation and elimination.
(6) package insert.
(7) formulation report

I. Background:

1. NOVANTRONE contains mitoxantrone hydrochloride as active ingredient which is a synthetic anthracenedione. This drug has the following structural and empirical formula $C_{22}H_{28}N_4O_6.2HCl$ with a molecular weight of 517.4.



Mitoxantrone is a potent antineoplastic agent intended for intravenous use in the treatment of certain neoplastic diseases. It has a cytocidal effect in both proliferating and nonproliferating culture human cells, suggesting lack of specificity for any phase of the cell cycle. It inhibits DNA and RNA synthesis, induces nuclear aberrations, however, the mechanism of action has not been fully elucidated.

2. Formulations - During the development of NOVANTRONE two variations of the formulation (MRWO 724,480 and MRWO 724,868) were used in clinical studies. The composition of each formulation is listed in Table 1. Each formulation is iso-osmotic and has a pH of 3.5 + 0.5. Crystal formation was reported with MRWO 724,480 after protracted storage in some vials. Investigation into this problem revealed that the crystalline material was a sparingly soluble salt of the dithionate anion (formed by oxidation of metabisulfite anion in solution) and mitoxantrone cation. When the metabisulfite concentration was decreased (MRWO 724,868 formulation), the crystal formation was eliminated. The acetate buffer system was added to the MRWO 724,868 formulation to increase pH stability since the pH had been observed to occasionally drift in some vials prepared with formulation MRWO 724,480 in which no excipient buffer system was present. The intended marketed product, NOVANTRONE, is supplied as a sterile dark blue aqueous solution of the MRWO 724,668 formulation. However, all clinical pharmacokinetic studies submitted in the May 15, 1984 application were conducted using the MRWO 724,480 formulation.

Table 1

Ingredient	MRWO 724.480		MRWO 724,868 Marketed Product	
	mg/ml	% w/v	mg/ml	% w/v
Mitoxantrone HCl*	2.0	0.2	2.0	0.2
Sodium Chloride, USP				
Sodium Metabisulfite, Reagent Anhydrous				
Sodium Acetate, Reagent Anhydrous				
Acetic Acid, Glacial				
Water for Injection, USP	qs ad			

* Expressed as the free base.
** Concentration as formulated; consumed during the manufacturing process and not detectable in finished vials.

3. The sponsor resubmitted a formulation report on October 21, 1985. The report provides clinical assessment of these two formulations on myelosuppression and the disease response rate.

This review contains an evaluation of the following:

(1) 4 clinical pharmacokinetic studies (Report #40, 41, 39, and 42).
(2) 3 clinical pharmacokinetic studies from the literature (Report# 36, 38 and 37).
(3) 1 interim report on clinical pharmacokinetics of mitoxantrone - multi-center studies for terminal half-life of the drug using RIA (Report #44).
(4) 1 metabolism study in human (Report #54).
(5) 2 in vitro protein binding studies (Report #58 and 59).
(6) 4 animal studies related to the drug's tissue distribution, accumulation and elimination (Report #33, 25, 47, and 50).
(7) package insert.
(8) formulation report submitted on October 21, 1985.

Note: The report numbers refer to the report numbers in the preclinical registration package.

II. Summary of Human Studies (Results and Comments)

1. Pharmacokinetics

IV administered mitoxantrone disappears from plasma with multiexponential kinetics. Seven pharmacokinetic studies in cancer patients have been conducted; three of which report biphasic (Report #36, #37 and #38) and four of which report triphasic kinetics (Report #40, 41, 39, and 42). Those reporting biphasic kinetics lack either sufficient early or late collected blood to adequately describe the kinetics. Variability in the estimated pharmacokinetic parameters in these studies is due to differences in the number and times of blood sample collection and the sensitivity limitations of the HPLC analytical procedures. Large intersubject variability within studies may also reflect the clinical condition of the tested patients. Viewed collectively, all studies indicate a rapid initial distribution phase(s) followed by a relatively slow elimination phase. The terminal half-life of the drug has been more recently re-estimated using a more sensitive RIA procedure.

(a) Table 2 summarizes the parameters of interest in these studies.

Table 2

ators	No. of patients and dosage	mean half-life	CL$_T$	Vdr	Method (sensitivity)
#40)	N=5 12 mg/m^2	*t1/2 α = 6 min. t1/2 β = 62 min. t1/2 γ = 38.6 hr	0.57 L/min/m^2	1875 L/m^2	HPLC (ng/ml)
#41)	N=25 (15 normal 7 hepatic disfunction 3 renal disfunction) 6-12 mg/m^2	*t1/2 α = 2.35 min t1/2 β = 16.6 min t1/2 γ = 3.27 hr	0.315 L/min/m^2	87.9 L/m^2	HPLC (ng/ml)
#39)	N=17 (pediatric & young adult, 11 normal, 7 hepatic disfunction 12-20 mg/m^2	*t1/2 α = 3.60 min t1/2 β = 25.2 min t1/2 γ = 2.86 hr	* 0.471 L/min/m^2	* 168.1 L/m^2	HPLC (ng/ml)
#42)	N=3 24 mg/m^2	t1/2 α = 4.1 min t1/2 β = 19.8 min t1/2 γ = 8.9 hr	0.37 L/m^2	317 L/m^2	HPLC (ng/ml)
#38)	N=6 12 mg/m^2	t1/2 β =56 min	--	--	HPLC
#36	N=6 (see Report #37) N=5 (abnormal liver function or third space) 2-4 mg/m^2 (1 at 12 mg/m^2)	**t1/2α =23.9 min (11.5 - 63.6) t1/2 β = 70.7 hr (53.3 - 173.2)	100.7 ml/kg/hr	11.4 L/kg	HPLC
#37)	N=6 1 - 3 mg/m^2	**t1/2 α = 13.7 min t1/2 β = 37.4 hr	238.7 ml/kg/hr	13.8 L/kg	HPLC
er son -center #44)	N=6 14 mg/m^2 (single dose) or 7.5 mg/m^2(daily x5) 12 mg/m^2(daily x5	t1/2 = 12.4 days			RIA (pg/ml)

etric mean
monic mean

(b) The initial rapid distribution phase (t1/2 range 2.35-14 min.) appears to be due to the distribution of mitoxantrone into the formed elements of the blood. The distribution of the drug into tissues varies from 16.6 min. to 62 min. The terminal half-life has been estimated and varies from 1 to 38.6 hr by HPLC and 12.4 days by RIA. The longer elimination half-life indicated by RIA is consistent with the prolonged urinary excretion as well as the persistence of mitoxantrone and/or related materials in the major tissues of man.

2. Excretion

Mitoxantrone is excreted slowly via the renal and hepatobiliary systems with the latter predominating. In the 7 patients treated with ^{14}C-Mitoxantrone (12 mg/m^2) 13.6 to 24.8% (mean 18.3%) of the administered radioactivity was excreted in the feces within 5 days; 6.2 to 23.5% (mean 10.1%) was excreted in urine (55% of the urinary recovered ^{14}C was excreted during the first 24 hr of post infusion). Of the material recovered in the urine, 65% was unchanged mitoxantrone. Radioactivity was also found in the saliva samples of the patients. In the 6 patients with normal (dose = 1-3 mg/m^2) and the 5 patients with abnormal liver function (dose=2 - 12 mg/m^2) an average of 11.3 and 10.2% of the administered ^{14}C was excreted in urine in 72 hours (of which 64.4 and 50.0% was mitoxantrone, respectively). All data indicate that renal excretion is limited which is in accord with the results that CL_R contributes to only about 7% of the CL_T.

3. Metabolism

Dicarboxylic acid (major metabolite, A) and monocarboxylic acid (minor metabolite, B) derivatives of mitoxantrone (resulting from the oxidation of the terminal OH group) and their glucuronide conjugates were identified in the urine of two of the ten patients studied (Report #54). The results may suggest individual patient variation in mitoxantrone metabolism. Similar results were also observed in Alberts patients (Report #40). The urine excretion for patient # 002 (first course drug treatment) was 23.5% for total radioactivity and 7.9% for mitoxantrone (measured by HPLC). However, patients #005 and #007 who received prior mitoxantrone treatment showed little/no difference in the [^{14}C] and [mitoxantrone] recovered in the urine. Further analysis of the urine samples from these two patients indicated the two polar metabolites only existed as minor constituents. It should be noted that the urine excretion pattern (metabolites vs. parent drug) of each of Albert's patients agrees with his/her plasma pattern (^{14}C vs. mitoxantrone).

4. Tissues Distribution and Accumulation

Mitoxantrone is rapidly cleared from the plasma by extensive sequestration into the tissues of cancer patients (as indicated by the large values of CL_T and estimated Vdr (Table 2). Re-entry of the drug into plasma is slow (small values of K_{31}). Autopsy specimens (11 tissues) obtained from one patient at 35 days after a single I.V. dose of 12 mg ^{14}C-mitoxantrone/m^2 had from 78 (bone marrow) to 1140 (liver) ng of radioactivity/g confirming the distribution into and the persistence of drug and/or metabolite(s) in the major tissues in man (Report #40). An estimate of 15% of the administered dose was retained by the seven (heart, liver, kidney, lung, spleen, thyroid

and bone marrow) organs analyzed. The mean concentrations of radioactivity in the 11 tissues in man (dosed at 12 mg/m^2) and in the same tissues in rat (3.5 mg/m^2), dog (7.6 mg/m^2) and monkey (12 mg/m^2) at 30 or 35 days were 583, 317, 483, and 734 ng/g, respectively. These data suggest that the tissue concentrations and distribution among tissues in man and laboratory animals are similar. Based on the above information and the following animal data:

(1) Tissue concentrations of total radioactivity were proportional to dose (0.25-0.75 mg/kg in rat, and 0.05-0.2 mg/kg in dog).

(2) Concentrations in most tissues were one to three orders of magnitude greater than the plasma concentration.

(3) Concentrations of radioactivity in all tissues declined monoexponentially with a mean half-life of 20-25 days in rat (49 days in dog) while radioactivity in serum disappeared more rapidly with a terminal half-life of 11.3 days (36 days in dog's blood).

(4) There was an absence of any detectable metabolites in most tissues (except liver, kidney and serum).

The half-life for the parent drug in rat tissue is 2 times of that measured in plasma by RIA. Therefore, it is reasonable to assume that the half-life of mitoxantrone in human tissues may be longer than 12.4 days which was estimated from plasma data by using the same RIA procedure that was used for the rat study. From this extrapolation data and the results of Report #40 (excretion and autopsy data) it is highly probable that this drug would accumulate in the body under a dosing schedule of 12 mg/m^2 given every 3 weeks (the proposed dosage regimen in the labeling is 14 mg/m^2 given every 3 weeks). Additionally, an effect of drug accumulation on the pharmacokinetics and metabolism of mitoxantrone is suspected but it can not be concluded with the limited information provided in this submission (See Report #40 Deficiencies (2)).

5. Protein Binding

Mitoxantrone is 78.3% bound to human plasma proteins in the concentration range of 26 to 455 ng/ml. The extent of binding is independent of concentration and was not affected by the presence of diphenyhydantoin, doxorubicin, methotrexate, prednisone, prednisolone, heparin and acetylsalicylic acid at their maximum reported therapeutic concentrations. Human albumin and α_1-acid glycoprotein bound 76 and 66% of the mitoxantrone when dialysed against solutions of 50, 200 and 500 ng/ml.

6. Effects of hepatic disfunction on drug's pharmacokinetics

Report #41 suggested that the pharmacokinetics of mitoxantrone in patients with abnormal liver functions (N=[illegible]) was not significantly different from patients with normal liver function (N=15). However, Report #36 found that patients with abnormal liver function (N=5) had a CL_T less than one half of that observed in patients (N=6) with normal liver function (100.7 vs. 238.7 ml/kg/hr). The terminal $t_{1/2}$ of the drug was almost double in those patients with liver disfunction (70.7 vs. 37.4 hr). Due to (1) the reliability of the pharmacokinetic parameters estimated in Report #41 as being questionable (see Report #41 Deficiencies), and (2) a lack of individual plasma data for an accurate evaluation of Report #36, no conclusion on this issue can be made at this time.

III. Individual Reports (Reviewed in Detail)

A. Report #40

1. Title: "Distribution and elimination of ^{14}C-labeled NOVANTRONE (Mitoxantrone.HCl, CL 232,315) following a single intravenous dose in cancer patients."

2. Investigator: David S. Alberts, M.D., University of Arizona, Tucson, Arizona.

3. Objective: To determine the distribu ion and elimination of ^{14}C-labeled NOVANTRONE after a single intravenous dose of 12 mg/m^2 in cancer patients.

4. Formulation: 10.5 ml sterile solution contains:
Active ingredient - ^{14}C (side chain) labelled mitoxantrone.2HCl, 2 mg as free base/ml, specific activity 8.85 mcCi/mg.

Inactive ingredients - sodium metabisulfite 0.2% w/v, sodium chloride 0.8% w/v.

5. Study Design and Procedures: 8 terminally ill patients (5 females, 3 males, age 25-74 years) participated in this phase I open-label study. 5 patients received the drug as the first course treatment. 2 had received 2 previous courses at triweekly intervals, and 1 had received 12 previous courses on the same schedule. NOVANTRONE was administered (12 mg/m^2) as a constant intravenous infusion over 30-35 min. Blood samples (0-96 hr, 7 patients only), urine (0-120 hr), total fecal materials (5 days), saliva samples were collected. Biopsies of the disease involved organs were obtained 5-22 hrs after dosing and autopsy specimens were obtained 35 days after drug administration.

6. Analytical Procedures:

(1) ^{14}C content (mitoxantrone and related materials) in plasma, urine, fecal material, blood formed elements, saliva, tissues removed at biopsies, and in one case at autopsy was assessed by liquid scintillation counting.

(2) Concentration of mitoxantrone in plasma and urine was determined by HPLC using external standard methodology (sensitivity of the assay 1 ng/ml). Prior to HPLC analysis ascorbic acid was added to plasma for maintaining sample integrity. A VAC-ELUT^{TM} system equipped with BOND-ELUT^{TM} one ml C18 cartridge was employed for sample cleanup purposes.

7. Study Results:

(1) Plasma mitoxantrone and radioactivity (^{14}C) decreased rapidly during the first 1-2 hrs after infusion completion. Thereafter the decrease was much slower reaching a point after about 12 hrs where the decrease was quite slow. Plasma ^{14}C decreased in two different patterns. In one group of 3

patients (#001, 002, 004), the plasma ^{14}C remained approximately equal to the plasma mitoxantrone for 5-20 min, but then the two concentrations began to diverge reaching a concentration ratio of 5:1 (^{14}C/mitoxantrone). In the second group (#005, 006, and 007), the decline of plasma ^{14}C concentration paralleled that of the plasma mitoxantrone with little difference between the two concentrations.

(2) The pharmacokinetic parameters in 5 of the 7 patients studied were best described by a three-compartment open model (Table 40.1, $t_{1/2}\alpha$ = 0.1 hr, $t_{1/2}\beta$ = 1.03 hr, and $t_{1/2}\gamma$ = 38.6 hrs) while the data from the remaining 2 patients fit a two compartment model (these two patients #004 and 005 were not included in the pharmacokinetic analysis). Individual data are listed in table 40.4.

(3) The ratio of blood formed elements (FE)-^{14}C/plasma-^{14}C ranged between 2:1 and 10:1.

(4) Recovery of drug-related material in urine was limited over a 5-day period. 6.5% (range [redacted]) of the administered dose was recovered as mitoxantrone while 10.1% (range [redacted]) was accounted for as mitoxantrone plus related material (Table 40.2). The majority of the material was excreted during the first 24 hrs post infusion (90% of the mitoxantrone and 55% of the ^{14}C-radioactivity). Mean renal clearance (CL_R=70 ml/min) was 7.6% of the total body clearance. Three metabolites were detected in urine but were not yet identified.

(5) Mean fecal recovery of ^{14}C-material in the 5 patients who had more than one bowel movement during the 5 days collection period was 18.3% (range [redacted]).

(6) ^{14}C-material was found in saliva samples taken at 96 hr (4.0 ng mitoxantrone equivalents/ml) and 120 hr (3.6 ng drug eq/ml).

(7) Tissue distribution of mitoxantrone and related materials is wide and extensive. This is demonstrated by (a) a large mean apparent volume of distribution (Vdr=1875 l/m^2), (b) a very small value of K_{31}, (c) biopsy specimens of malignant tissue taken 5-22 hrs after dosing indicated uptake of ^{14}C in all tumors examined (Table 40.3), and (d) all specimens taken at autopsy (patient #002) 35 days after drug administration contained measurable quanitities of ^{14}C material (Table 40.3). Highly perfused tissues attained higher concentration of drug related material. An estimate of 15% of the administered dose was retained by the seven organs analyzed.

Both the autopsy data and the excretion data (urine plus fecal, 28% of the administered dose recovered in 5 days) suggest that mitoxantrone and/or ^{14}C-related material has a very long half life which is considerably longer than the t1/2 γ= 38.6 hr determined in this study.

8. Deficiencies:

(1) Inconclusive evidence with regard to the effect of mitoxantrone accumulation on the pharmacokinetics and metabolism of the drug.

From this study #40, it is evident that the drug and/or related materials probably would accumulate in the body on a dosing schedule of 12 mg/m^2 every 3 weeks. The investigator claimed that repeated dosing for as many as 12 courses (patient #007) had no noticeable effect on the calculated pharmacokinetic parameters (Table 40.4). The conclusion is less convincing without further support due to the following reasons:

a. Lack of a well controlled study for patient #007 multiple dose data).

b. Only limited number of terminally ill patients with various disease states were analyzed (N=5).

c. Variability of individual's pharmacokinetic data.

Furthermore, in analyzing the pattern of plasma disappearance of ^{14}C/mitoxantrone for the 7 patients studied (Appendix I and II), it was found that 3 patients (#005, #006, #007) who had prior mitoxantrone treatment fell into one (Group II) of patients whose decline of the plasma ^{14}C concentrations paralleled the decline of plasma mitoxantrone with little difference between the two determined concentrations, while those patients who received the drug for the first time had a slower decline in plasma levels of ^{14}C and had a final [^{14}C]/[mitoxantrone] concentration ratio close to 5:1 (Group I). The urine excretion patterns (^{14}C vs mitoxantrone) of each patient agreed with his/her plasma data. Of particular interest was the finding for patient #007 who had 12 prior mitoxantrone treatments. The total amount of mitoxantrone recovered in each urine collection period for this patient equaled the amount of total ^{14}C recovered per sample suggesting that no metabolites were present. Whether these observations imply alteration of mitoxantrone metabolism during the course of repeated dosing or simply reflect common characteristics of these 3 patients can not be concluded from the data reported in this study. It is recommended that the sponsor submit available information or conduct a multiple dosing study to clarify this issue.

2. Underestimated elimination half-life.

The autopsy data (at least 15% of the administered radioactivity remained in the body 35 days after dosing) and the excretion data (only 20-31% of the drug was recovered in 5 days) suggest that mitoxantrone and/or related materials should have a considerably longer elimination half-life than the presently reported values ($t_{1/2\beta}$=20.8-69.3 hrs) which were derived from plasma data obtained near the assay's lower limit of sensitivity (ng/ml).

3. Lack of assay validation data.

In this study, (#40) an external standard method of analysis of mitoxantrone was used to determine the concentration of mitoxantrone in plasma and urine (i.e. by comparing the peak height of mitoxantrone of patient's samples to that of the spiked samples obtained from the HPLC chromatograms which were run separately). The following assay validation data for the sponsor's HPLC procedure should be provided:

a. Recovery for mitoxantrone from urine.
b. Standard calibration curves for mitoxantrone from plasma and urine.
c. Intraday and interday reproducibilities of these standard calibration curves.

d. Precession of assay demonstrated by assaying patient's urine samples at several levels of drug concentration.
e. Some HPLC profiles of patient's plasma and urine samples at different levels of drug concentration.

Due to lack of assay validation data, the pharmacokinetic parameters obtained by the HPLC method cannot be accurately evaluated at this time.

9. Conclusion:

The Division of Biopharmaceutics finds the pharmacokinetic parameters obtained questionable and not acceptable at this time due to (1) a lack of HPLC assay validation data and (2) other data that suggest the elimination half-life of the study drug to be much longer than the study's reported value ($t_{1/2}$ = 38.6 hr). The study's tissue distribution data for ^{14}C mitoxantrone is however found to be acceptable. This study demonstrated that mitoxantrone is distributed widely and extensively into the tissues of man.

TABLE 40.1

Mean Mitoxantrone Pharmacokinetic Parameters

N = 5

Parameter	Mean	Standard Deviation	Min.	Max.	Standard Error Mean	C.V.%
α /hr	7.33	2.38			1.0	32.5
β /hr	0.687	0.170			0.076	24.8
γ /hr	0.0199	0.0101			0.0045	50.6
$t_{1/2}^{\alpha}$ hr	0.099					38.8
$t_{1/2}^{\beta}$ hr	1.03					26.0
$t_{1/2}^{\gamma}$ hr	38.6					50.7
K_{12} /hr	1.7	1.0			0.46	60.4
K_{21} /hr	0.94	0.28			0.12	30.4
K_{13} /hr	1.8	0.55			0.24	30.3
K_{31} /hr	0.0324	0.0131			0.0059	40.2
K_{10} /hr	3.5	1.7			0.79	50.5
V_1 L/m^2	10.34					66.3
V_2 L/m^2	16.78					66.2
V_3 L/m^2	612.					63.7
VD_{SS} L/m^2	641.					63.3
VD_{γ} L/m^2	1875.	670.			299.	35.7
CL_T L/min/m^2	0.57	0.24			0.10	42.2
CL_R ml/min	70.	33.			14.	47.6

The following values are geometric means:

$T_{1/2}$	All values
V_1	
V_2	
V_3	
VD_{SS}	

TABLE 4i.2

Mean Urinary Recovery of Mitoxantrone and Mitoxantrone-Related Material

Time Hr.	N	Percent of Administered Dose: Related C-14 Material	N	Mitoxantrone
0-4	6	2.9	6	3.7
4-8	6	1.2	6	1.2
8-16	5	.61	5	0.36
16-24	5	0.80	5	0.49
0-24	7	5.6	7	5.9
24-48	7	1.4	6	0.50
48-72	7	0.95	7	0.26
72-96	7	0.82	6	0.33
96-120	4	0.86	4	0.21
Average Total Recovery 0-120 Hours		10.1		6.5
		Range (6.2-23.8)		(5.2-7.9)

TABLE 4.3

^{14}C-Labelled Mitoxantrone Related Material in Biopsy and Autopsy Specimens

Patient No.	Tissue	Time of Sample	Concentration* ng mitoxantrone equivalents/$1x10^6$ cells
002	Bone Marrow	6 hr	
	Whole		0.06
	Red cells only		0.045
	White cells only		1.13
003	Squamous Cell	5 hr 15 min	0.059
004	Lymph Node	5 hr	1.32
008	Melanoma nodule	22 hr 15 min	0.034
			ng mitoxantrone equivalents/g wet weight
002	Heart	35 days	716.
	Liver		1140.
	Bone Marrow		78.
	Lung		276.
	Thyroid		958.
	Kidney		312.
	Spleen		733.
	Pancreas		1040.
	Stomach		855.
	Small Intestine		173.
	Lymph Node		432.

* $1x10^6$ cells is = equal to 1 mg.

Table 40.4

NOVANTRONE®

Individual Pharmacokinetic Parameters

Dose 12 mg/m²

Patient #	α hr^{-1}	β hr^{-1}	γ hr^{-1}	$t_{\frac{1}{2}}\alpha$ hr	$t_{\frac{1}{2}}\beta$ hr	$t_{\frac{1}{2}}\gamma$ hr	Cl_T L/m²	Cl_R ml/min
(1) 001								
(1) 002								
(1) 003								
(2) 006								
(17) 007								

Patient #	k_{12} hr^{-1}	k_{21} hr^{-1}	k_{13} hr^{-1}	k_{31} hr^{-1}	k_{10} hr^{-1}	V_1 L/m²	V_2 L/m²	V_3 L/m²	Vd_{ss} L/m²	Vd_γ L/m²
001										
002										
003										
006										
007										

Appendix I Group I Plasma ^{14}C and mitoxantrone concentrations (patients who had no prior mitoxantrone treatment)

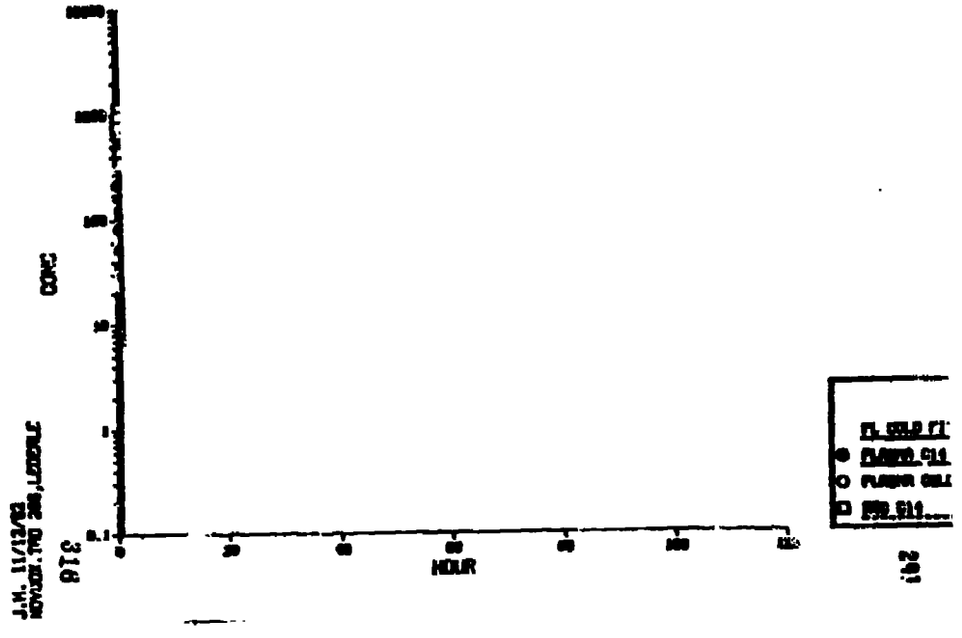
PATIENT NO. 001

SUBJECT 1
DOSE 17 MG, 12 MG/M2

Infusion Time 30 Minutes
Total Dose 17 mg

Time Hour	Time Min.	Plasma C-14 ng mitoxantrone equivalents/ml	Plasma ng mitoxantrone/ml	RBC C-14 ng mitoxantrone equivalents/ml
	-10			
	-5			
	2			
	4			
	6			
	10			
	15			
	30			
	45			
1				
2				
4	15			
6	30			
11	30			
24				
36				
48				
72				
96				
120	45			

- No data for point.



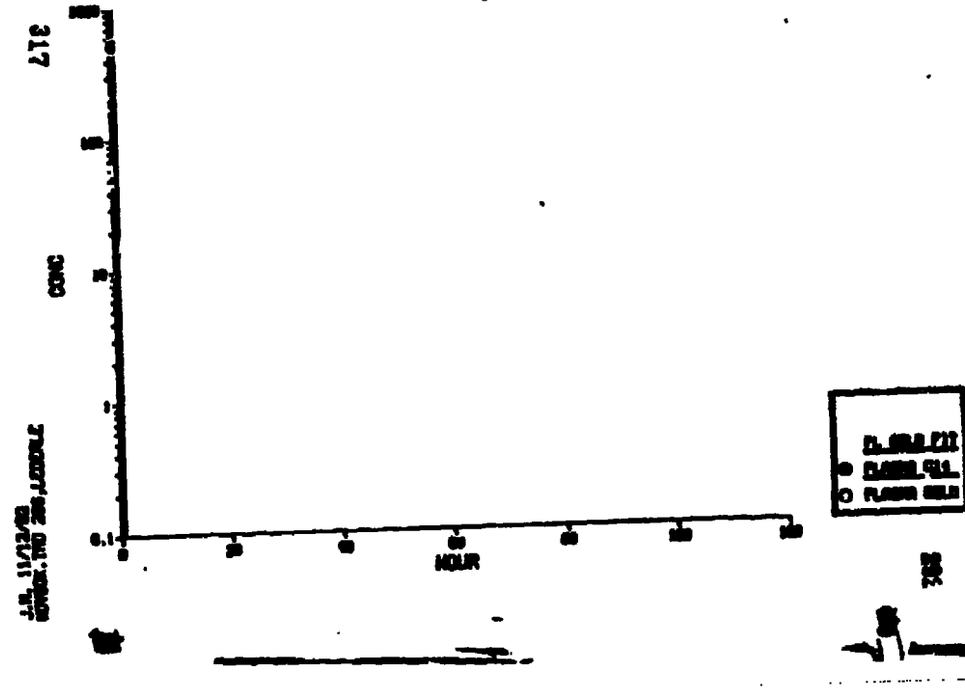
PATIENT No. 002

SUBJECT 2
DOSE 16 MG, ~~16~~ 12 MG/M2

Infusion Time 30 Minutes
Total Dose 16 mg

Time Hour	Time Min.	Plasma C-14 ng mitoxantrone equivalents/ml	Plasma ng mitoxantrone/ml	RBC C-14 ng mitoxantrone equivalents/ml
	-5			
	5			
	15			
	30			
1				
2				
3				
4				
5				
6	30			
8				
11				
23				
35				
47				
72				

- No data for point.



Appendix I (cont'd)

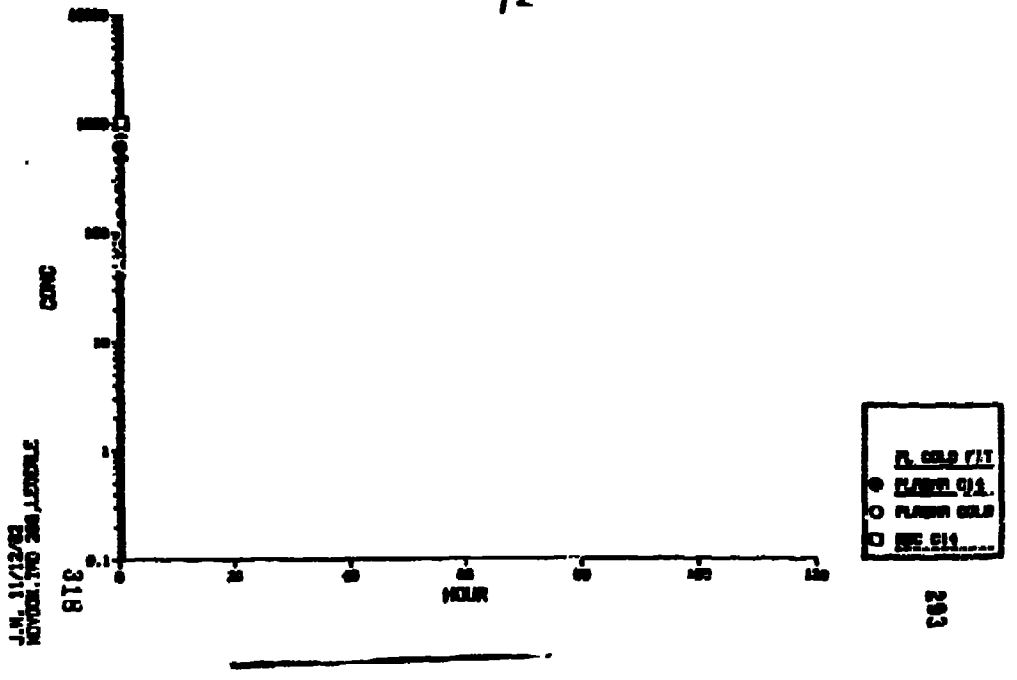
SUBJECT 3
DOSE 23 MG, ~~18~~ MG/M2
12

PATIENT NO. 003

Infusion Time 30 minutes
Total Dose 23 mg

Time Hour	Time Min.	Plasma C-14 ng eq/ml	Plasma ng eq/ml	RBC C-14 ng eq/ml
	-10			
	- 5			
	2			
	3			
	6			
	10			
	15			
	20			
	30			
	45			
1				
2	5			
4	10			
6	20			
8	15			
11	5			
25				
34	10			
48				
72	25			
96				

- No data for point.



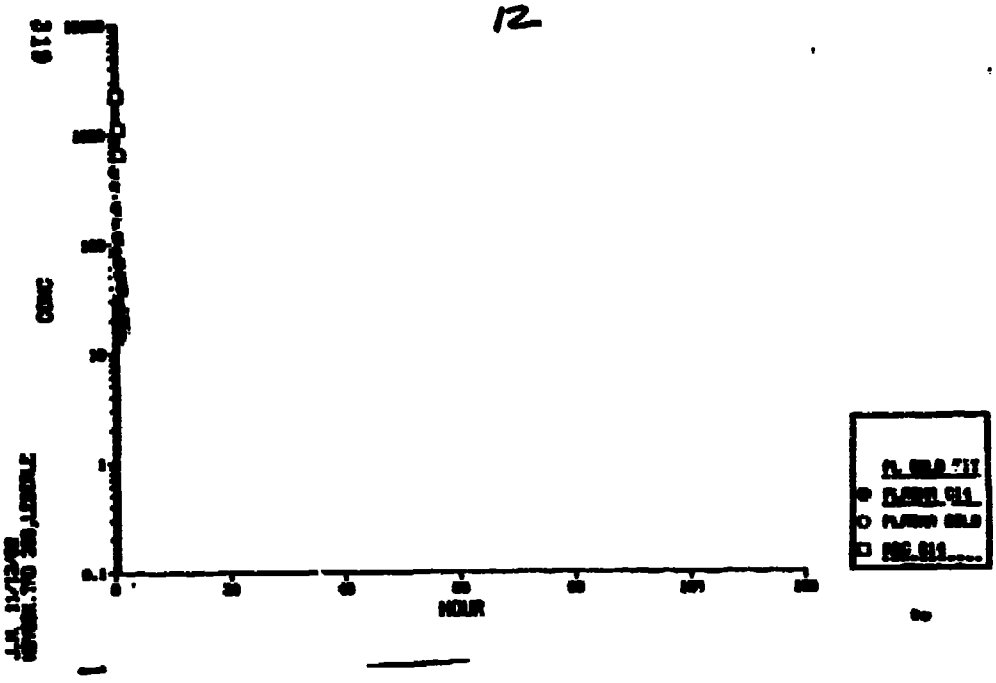
SUBJECT 4
DOSE 18 MG, ~~18~~ MG/M2
12

PATIENT NO. 004

Infusion Time 30 minutes
Total Dose 18 mg

Time Hour	Time Min.	Plasma C-14 ng eq/ml	Plasma ng eq/ml	RBC C-14 ng eq/ml
	-10			
	- 5			
	13			
	25			
	30			
	45			
1				
2				
4	5			
7	10			
11	20			
15				
25	30*			
35	45			
71	40			
95				

* Sample hemolyzed.
- No data for point.



PATIENT NO. 005

Infusion Time 60 Minutes
Total Dose 22 mg

Time Hour	Time Min.	Plasma C-14 ng mitoxantrone equivalents/ml	Plasma ng mitoxantrone/ml	RBC C-14 ng mitoxantrone equivalents/ml
	-10			
	1			
	5			
	10			
	23			
	32			
	47			
1	5			
2				
3				
4				
5				
6	3			
7				
11	35			
27	35			
33	65			
49	65			
96	30			

PATIENT NO. 006

Infusion Time 30 Minutes
Total Dose 21 mg

Time Hour	Time Min.	Plasma C-14 ng mitoxantrone equivalents/ml	Plasma ng mitoxantrone/ml	RBC C-14 ng mitoxantrone equivalents/ml
	-15			
	- 5			
	3			
	15			
	30			
	45			
1				
2	5			
3	5			
4	5			
5				
6				
7				
8	5			
11	25			
24	15			
25				
48				
804				
1008				

PATIENT NO. 007

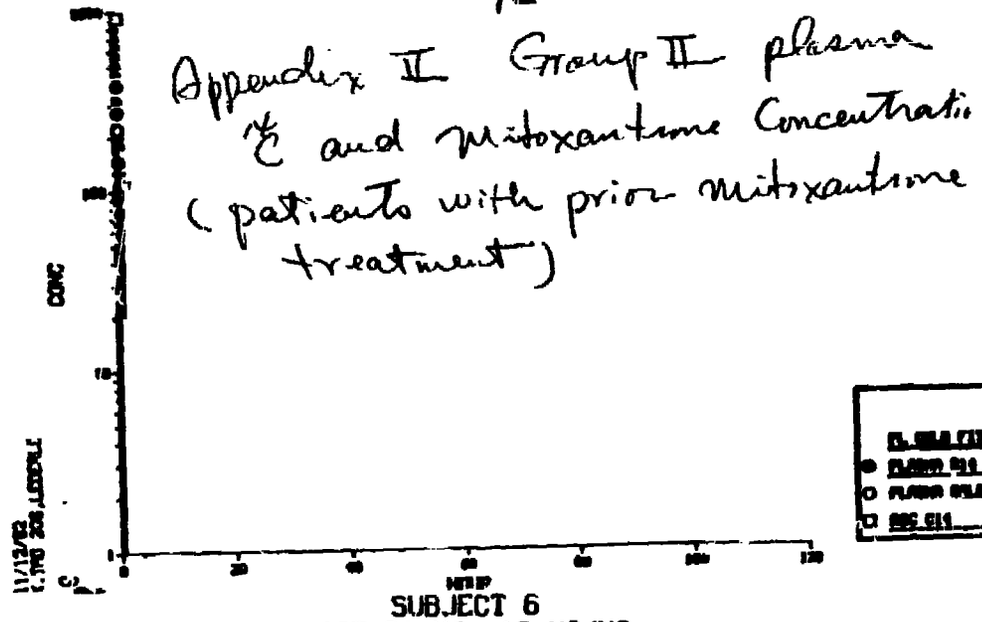
Infusion Time 32 Minutes
Total Dose 22 mg

Time Hour	Time Min.	Plasma C-14 ng mitoxantrone equivalents/ml	Plasma ng mitoxantrone/ml	RBC C-14 ng mitoxantrone equivalents/ml
	- 5			
	1			
	4			
	8			
	11			
	17			
	30			
	48			
1				
1	05			
3	30			
4	3			
5				
6	30			
8				
9	45			
11				
22	55			
24				
48	40			
72				

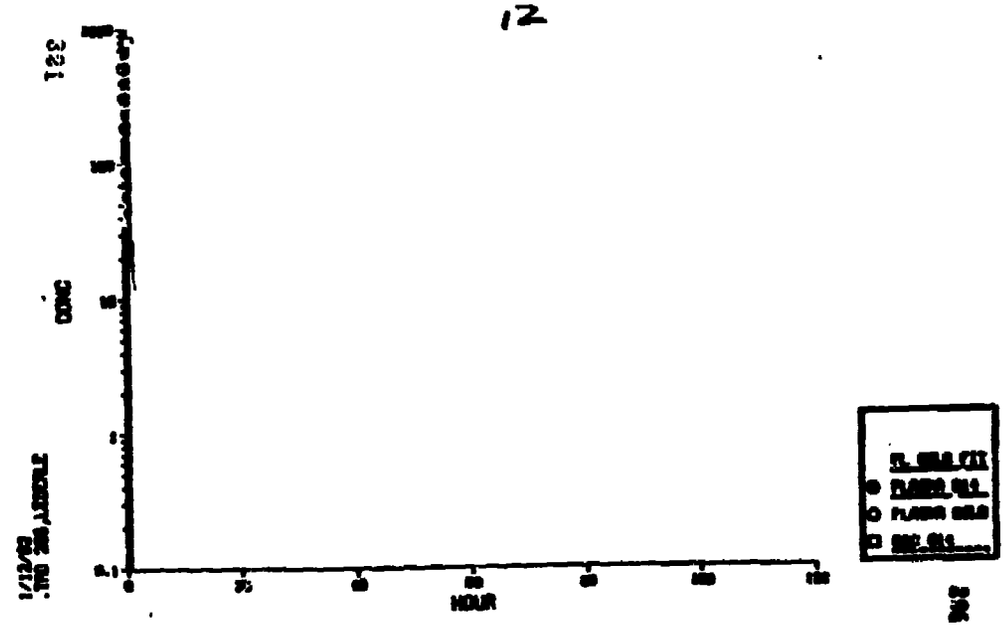
- No data for this point.

SUBJECT 5
DOSE 22 MG, ~~16~~ MG/M2
12

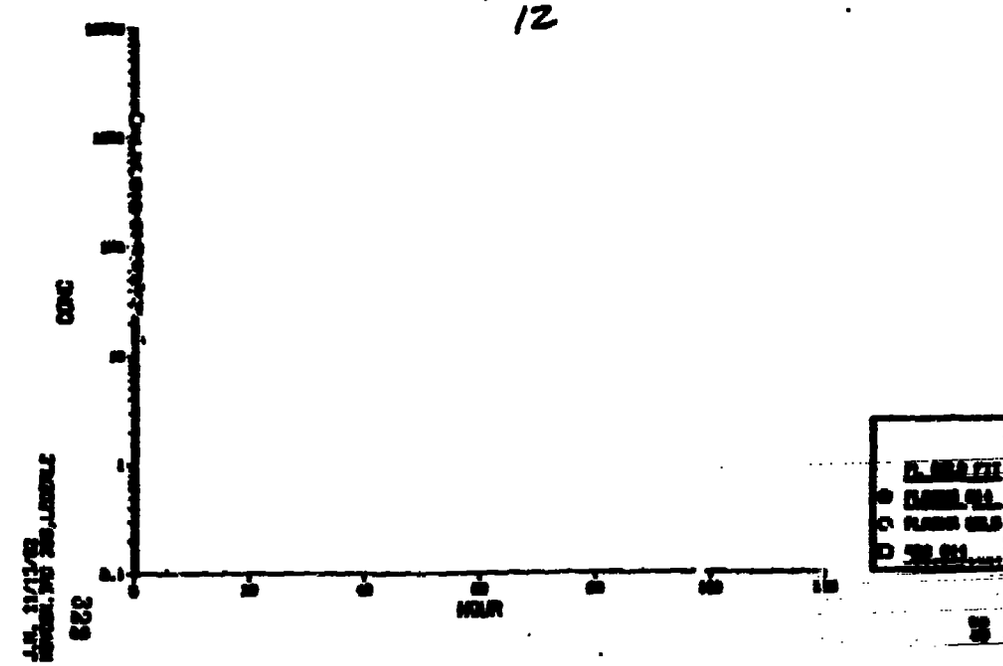
Appendix II Group II plasma & and Mitoxantrone Concentrations (patients with prior mitoxantrone treatment)



SUBJECT 6
DOSE 21 MG, ~~42~~ MG/M2
12



SUBJECT 7
DOSE 22 MG, ~~44~~ MG/M2
12



B. Report #41

1. Title:

"Pharmacokinetics of NOVANTRONE (Mitoxantrone.HCl, CL 232,315) following administration of a single intravenous dose"

2. Investigators:

Louis Malspeis, Ph.D.
James A. Neidhart, M.D.
Ohio State University, Columbus, Ohio

3. Objective:

To assess and describe the pharmacokinetics of NOVANTRONE in cancer patients under a phase I clinical pharmacology study.

4. Formulation:

10 ml sterile solution contains:

active ingredient	mitoxantrone.2HCl	0.5 mg free base/ml
inactive ingredient	sodium metabisulfite	
	sodium chloride	

5. Study Design & Procedure:

This study was an open-label rising dose tolerance study in 25 terminally ill patients (11 men and 14 women, age 17-70 years). Single doses of 6 mg/m^2 (N=16), 7 mg/m^2 (N=1), 8 mg/m^2 (N=1), or 12 mg/m^2 (N=7) of NOVANTRONE were given as constant intravenous infusions over 3-32 minutes. Blood (0-72 hr), and urine (24-48, 48-72 hr) samples were collected.

6. Analytical Method:

Plasma and urine samples were first extracted using dichloromethane and potassium carbonate followed by an (extraction efficiency was 67.4%).
was included as internal standard. The resulting sample was then analyzed by HPLC (assay sensitivity ng/ml).

7. Results:

(1) The plasma mitoxantrone concentration-time data were fitted to a 3 compartment model (Table 41.1; $t_{1/2\alpha}$=2.35 min, $t_{1/2\beta}$=16.6 min, $t_{1/2\gamma}$=3.27 hrs). However, the presence of urine mitoxantrone at 50 ng/ml (24-48 hr) and 25 ng/ml (48-72 hrs) suggests a much longer terminal half-life than the presently reported value of 3.27 hrs.

2. The large apparent volume of distribution (Vdr =87.9 l/m^2) could suggest that mitoxantrone is highly bound to tissue. The small value of K_{31}=0.0046 min^{-1} suggests the drug's re-entry to plasma is very slow.

(3) A comparison (by T-test) of the pharmacokinetic parameters for patients receiving 6 mg/m^2 (N=16) with those receiving 12 mg/m^2 (N=7) failed to show any dose dependency.

(4) Renal clearance (7 ml/min/m^2) estimated for two patients (1 normal, 1 liver disfunction) only contributes to 5% of the total body clearance. A comparison of the mean pharmacokinetic parameters of patients having normal renal function (N=15) with patients having renal impairment (N=3) (table 41.2) showed no significant difference. However, it was acknowledged by the investigator that 3 patients with various degrees of renal impairment constituted only a small sample.

(5) A comparison (T-test) of the mean pharmacokinetic parameters of patients having normal liver functions (N=15) with patients having hepatic disfunction (N=7) showed no significant differences. (Table 41.3). Individual data are listed in Table 41.4.

3. Deficiencies:

(1) Inadequate estimation of pharmacokinetic parameters.

Due to limitations of the assay the pharmacokinetic parameters estimated in this study were derived from plasma level data obtained for a period only covering 0-10 hr. Furthermore, 75% of the plasma samples collected at time points greater than 4 hrs after dosing for the 6 or 7 mg/m^2 doses had drug concentrations that were in a range where no information for extraction efficiency was given. Therefore, the reliability of the parameters reported in this study is questionable. Example, the estimated mean terminal half-life is 3.27h which is only 1/10 the value reported by Alberts (Report #40; 38.6 hr by HPLC), and by Savaraj (Report #36; 37.4 hr by HPLC). Besides the inconsistency with the results obtained from other studies, the estimated half life is also in conflict with the investigator's collected urine data which indicated the presence of mitoxantrone in 24-48 hr (50 ng/ml) and 48-72 hr samples (25 ng/ml). If indeed the terminal half-life was only 3.27 hrs, no drug should have been found in urine after 35 hrs (ten half-lives). By summing up their plasma data and urine data the investigators suggest a four compartment model may be more appropriate for this drug. Based on (1) the questionable parameters at hand, and (2) the lack of analysis of complete set of plasma samples (0-72 hr), the Division of Biopharmaceutics finds that the interpolation of the urine data into a not yet defined model is inadequate.

(2) Conflicting information with regard to the effects of hepatic disfunction on drug's pharmacokinetics.

This study also showed that the mean pharmacokinetic parameters obtained from patients with hepatic disfunction were not significantly different from those obtained for patients with normal liver function. However, the authors in Report #36 (literature article) found that 1) the former type patients had a total clearance that was less than one half that observed for patients with normal liver function (100.7 ml/kg/hr vs 238.7 ml/kg/hr), and 2) the terminal half-life of the drug was almost double (70.7 hr vs 37.4 hr) in those patients with hepatic dysfunction. Since the reliability of the pharmacokinetic parameters estimated in this study is questionable (Deficiency (1)) and Report #36 is a literature article that lacks individual data for a meaningful evaluation, no conclusion on this issue can be made at this time.

9. Conclusion:

The Division of Biopharmacuetics finds the pharmacokinetic parameters reported in this study not acceptable. However, the following information obtained in this study could be qualitatively used as supportive data of other studies.

(1) Mitoxantrone appears to be bound to tissues and re-entry of the drug into plasma is very slow.

(2) Renal elimination of the drug is minimal.

TABLE 41.1

Mean Mitoxantrone Pharmacokinetic Parameters (N=25)

Parameter	Units	Mean*	S.D.%	Min	Max
α	min^{-1}	0.2942	76.2	0.09850	1.10000
β	min^{-1}	0.04183	35.8	0.01650	0.06190
γ	min^{-1}	0.0035	41.0	0.00199	0.0087
$t_{1/2}^{\alpha}$	min	2.35	52.8	0.63	7.04
$t_{1/2}^{\beta}$	min	16.6	24.8	11.20	42.01
$t_{1/2}^{\gamma}$	hr	3.27	28.4	1.41	5.81
k_{12}	min^{-1}	0.0927	94.0	0.01376	0.31935
k_{21}	min^{-1}	0.0722	50.8	0.02063	0.19882
k_{13}	min^{-1}	0.0355	58.0	0.01035	0.10087
k_{31}	min^{-1}	0.0046	41.0	0.00239	0.00995
k_{10}	min^{-1}	0.1443	69.9	0.04541	0.77254
V_1	L/m^2	2.18	76.9	0.27	9.20
V_2	L/m^2	2.81	51.7	0.74	7.89
V_3	L/m^2	16.8	61.8	4.42	47.12
Vd_{ss}	L/m^2	22.5	55.8	8.19	58.47
Vd_{γ}	L/m^2	87.9	49.2	42.80	265.19
Cl	$L/min/m^2$	0.315	39.6	0.14	0.62

* Geometric mean.

TABLE 44.2

Comparison of the Mean Mitoxantrone Pharmacokinetic Parameters of "Normal" Patients with Patients Having Renal Dysfunction

Parameter		Normal N = 15		Renal Dysfunction N = 3		R**	95% Confidence Limits of R %	
		Mean*	C.V.%	Mean	C.V.%	%	Lower	Upper
α	(min^{-1})	0.2446	76.3	0.1896	75.3	77.5	48	125
β	(min^{-1})	0.0441	28.6	0.0375	62.1	85.0	40	181
γ	(min^{-1})	0.00348	44.6	0.00356	37.8	102.3	61	173
k_{12}	(min^{-1})	0.0942	96.9	0.0682	72.4	72.4	33	159
k_{21}	(min^{-1})	0.0783	48.4	0.0628	70.8	80.0	42	154
k_{13}	(min^{-1})	0.0448	92.1	0.0236	68.7	52.7	33	84
k_{31}	(min^{-1})	0.00472	45.9	0.00485	41.8	98.9	75	129
k_{10}	(min^{-1})	0.1426	67.1	0.0866	64.2	60.7	25	144
V_1	(L/m^2)	2.38	87.0	3.40	46.8	142.8	68	302
V_2	(L/m^2)	2.88	61.0	2.71	36.9	94.1	57	156
V_3	(L/m^2)	17.8	69.4	17.2	36.4	96.9	54	174
Vd_{ss}	(L/m^2)	20.9	95.2	23.8	29.6	113.7	60	214
Vd_γ	(L/m^2)	94.5	56.7	82.6	54.4	87.4	42	182
Cl_T	($L/min/m^2$)	0.358	55.3	0.295	38.8	82.4	47	145

* Geometric mean.

** R = Renal Dysfunction Value/Normal Value x 100.

TABLE 44.3

Comparison of the Mean Mitoxantrone Pharmacokinetic Parameters of "Normal" Patients with Patients Having Hepatic Dysfunction

Parameter		Normal N = 15		Hepatic Dysfunction N = 7		R**	95% Confidence Limits of R %	
		Mean*	C.V.%	Mean	C.V.%	%	Lower	Upper
α	(min^{-1})	0.2446	76.4	0.3747	65.4	153.2	80	295
β	(min^{-1})	0.0441	28.6	0.3910	41.7	88.7	62	127
γ	(min^{-1})	0.00348	44.6	0.00362	40.0	104	70	154
k_{12}	(min^{-1})	0.0942	96.9	0.1146	89.8	121	50	293
k_{21}	(min^{-1})	0.0783	82.1	0.0643	52.8	82.1	50	134
k_{13}	(min^{-1})	0.0448	92.	0.0366	49.8	81.7	44	156
k_{31}	(min^{-1})	0.00472	45.9	0.00448	31.8	94.9	67	135
k_{10}	(min^{-1})	0.1426	67.1	0.1804	66.2	129	58	243
V_1	(L/m^2)	2.38	87.0	1.52	51.0	63.9	34	118
V_2	(L/m^2)	2.88	61.0	2.69	40.5	93.4	59	149
V_3	(L/m^2)	17.8	69.4	12.3	71.0	69.6	27	177
Vd_{ss}	(L/m^2)	20.9	95.2	13.7	91.8	65.6	27	160
Vd_γ	(L/m^2)	94.5	56.7	77.2	29.4	81.6	56	120
Cl_T	($L/min/m^2$)	0.358	55.3	0.280	29.7	77.9	53	114

* Geometric mean.

** R = Hepatic Dysfunction Value/Normal Value x 100.

TABLE 41.4

SUMMARY OF MEAN (NSC 279836) MACROSCOPIC PHARMACOKINETIC PARAMETERS DURING A PHASE I CLINICAL STUDY

Patient	Dose (mg/m^2)	α (min^{-1})	β (min^{-1})	γ (min^{-1})	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)	$t_{1/2\gamma}$ (hours)	CL ($L/min/m^2$)
E.A.	6	0.285	0.0488	0.00462	2.43	14.20	2.50	0.430
J.A.	6	0.116	0.0165	0.00199	5.98	42.01	5.80	0.160
E.B.	6	0.585	0.0511	0.00249	1.18	13.56	4.63	0.179
S.B.	6	0.506	0.0505	0.00821	1.37	13.73	1.41	0.351
M.C.	6	0.159	0.0428	0.00264	4.36	15.13	4.38	0.409
W.E.	12	0.267	0.0348	0.00313	2.60	19.92	3.69	0.214
L.F.	6	0.270	0.0436	0.00391	2.57	15.90	2.95	0.353
R.G.	12	1.100	0.0619	0.00223	0.63	11.31	3.54	0.209
J.H.	12	0.381	0.0463	0.00515	1.82	14.97	2.24	0.620
M.H.	12	0.315	0.0619	0.00273	2.20	11.20	4.23	0.364
C.J.	12	0.132	0.0253	0.00217	5.25	27.40	5.34	0.352
R.J.	8	0.0450	0.0515	0.00409	1.54	13.46	2.82	0.221
B.L.	12	0.314	0.0251	0.00234	2.21	27.62	4.93	0.369
R.L.	12	0.099	0.0187	0.00315	7.00	37.07	3.66	0.192
D.M.	6	0.230	0.0393	0.00345	3.01	17.64	3.34	0.314
L.M.	6	0.663	0.0542	0.00440	1.05	11.71	2.62	0.306
J.P.	7	0.592	0.0525	0.00643	1.17	13.20	1.80	0.335
P.P.	6	0.141	0.0365	0.00246	4.92	18.99	4.69	0.137
J.R.	6	0.450	0.0432	0.00648	1.54	16.05	1.78	0.441
A.S.	6	0.707	0.0433	0.00472	0.98	16.01	2.45	0.323
B.S.	6	0.433	0.0616	0.00545	1.60	11.25	2.12	0.326
B.S.1	6	0.447	0.0415	0.00250	1.55	16.70	4.62	0.333
P.S.	6	0.631	0.0519	0.00315	1.10	13.36	3.67	0.479
A.T.	6	0.104	0.0342	0.00212	6.66	20.27	5.44	0.364
B.W.	6	0.370	0.0587	0.00589	1.87	11.81	1.96	0.289

TABLE 41.4 (Cont'd)

SUMMARY OF BRAD (NSC 279836) MICROSCOPIC PHARMACOKINETIC PARAMETERS DURING A PHASE I CLINICAL STUDY

Patient	Dose (mg/m^2)	k_{12} (min^{-1})	k_{21} (min^{-1})	k_{13} (min^{-1})	k_{31} (min^{-1})	k_{10} (min^{-1})
E.A.	6	0.08379	0.08160	0.04422	0.00643	0.12253
J.A.	6	0.02041	0.02063	0.02833	0.00296	0.08237
E.B.	6	0.25177	0.10724	0.05712	0.00317	0.219-1
S.B.	6	0.21137	0.10425	0.03736	0.00995	0.20217
H.C.	6	0.03035	0.06873	0.-1795	0.00320	0.08719
W.E.	12	0.08233	0.05691	0.-2135	0.00364	0.14031
L.F.	6	0.09024	0.08334	0.02298	0.00475	0.11806
R.G.	12	0.21194	0.07716	0.10087	0.00370	0.77254
J.H.	12	0.14072	0.19882	0.01941	0.00677	0.06741
H.H.	12	0.08419	0.10196	0.02867	0.00323	0.16191
C.J.	12	0.01413	0.02934	0.01035	0.00239	0.10277
E.J.	8	0.17473	0.10224	0.04253	0.00512	0.18125
E.L.	12	0.11462	0.04268	0.05478	0.00345	0.12364
E.L.	12	0.02669	0.02972	0.01416	0.00430	0.04341
E.M.	6	0.06573	0.06347	0.05123	0.00568	0.08638
L.M.	6	0.14845	0.07924	0.04837	0.00490	0.44572
J.P.	7	0.22297	0.09456	0.02793	0.00708	0.29891
P.P.	6	0.03048	0.05546	0.03649	0.00427	0.05362
J.R.	6	0.15408	0.07140	0.05824	0.00851	0.20714
A.S.	6	0.31935	0.08782	0.07050	0.00606	0.27136
B.S.	6	0.15e11	0.121e4	0.05147	0.00730	0.16388
B.S.1	6	0.15685	0.07052	0.06590	0.00338	0.19462
P.S.	6	0.26766	0.10501	0.04842	0.00376	0.26136
A.T.	6	0.01376	0.04429	0.01421	0.00261	0.06513
E.W.	6	0.13077	0.11874	0.05809	0.00915	0.1:789

TABLE 41.4 (cont'd)

SUMMARY OF DIAD (NSC 279836) VOLUME PHARMACOKINETIC PARAMETERS DURING A PHASE I CLINICAL STUDY

Patient	Dose (mg/m^2)	V_1 (L/m^2)	V_2 (L/m^2)	V_3 (L/m^2)	Vd_{ss} (L/m^2)	Vd_γ (L/m^2)
E.A.	6	3.51	3.60	24.13	31.23	93.03
J.A.	6	2.57	2.54	24.58	29.69	80.45
E.B.	6	0.82	1.92	14.73	17.47	71.6
S.B.	6	..74	3.52	6.52	11.78	42.80
M.C.	6	4.69	2.07	26.24	33.00	154.93
W.E.	12	1.52	2.20	9.00	12.73	68.17
L.F.	6	3.04	3.28	14.71	21.03	90.15
R.G.	12	0.27	0.74	7.37	8.38	63.90
J.H.	12	9.20	6.51	25.04	40.75	120.49
M.H.	12	3.48	2.88	30.92	37.27	206.57
C.J.	12	3.42	1.65	14.82	19.88	182.37
R.J.	8	1.28	2.18	10.60	14.05	94.46
B.L.	12	2.94	7.89	46.73	57.56	157.51
R.L.	12	4.23	3.80	13.96	21.99	60.96
B.M.	6	3.63	3.76	32.70	40.09	90.95
L.M.	6	0.685	1.28	6.77	8.74	69.99
J.P.	7	1.12	2.65	4.42	8.19	52.12
P.P.	6	2.55	1.40	21.79	25.74	55.52
J.R.	6	2.13	4.60	14.57	21.30	68.11
A.S.	6	1.19	4.33	13.83	19.34	68.55
E.S.	6	1.99	2.55	14.00	18.55	59.78
E.S.I	6	1.71	3.81	33.35	38.86	133.30
F.S.	6	1.83	4.67	23.61	30.11	152.13
A.T.	6	8.65	2.69	47.12	58.47	265.19
B.W.	6	2.45	2.70	15.35	20.69	68.98

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C. Report #39

1. Title:
"Pharmacokinetics of NOVANTRONE Mitoxantrone•HCl (CL 232, 15) following administration of a single intravenous dose in pediatric and young adult patients"

2. Investigvators:
James A. Neidhart, M.D.
Louis Malspeis, Ph.D.
James Miser, M.D.
Ohio State University, Columbus, Ohio

3. Objective:
To assess and describe the pharmacokinetics of NOVANTRONE in pediatric patients under a phase I clinical pharmacology study.

4. Formulation:
Same as Report #41

5. Study Design and Procedure:
This study was an open-label phase I rising-dose tolerance and safety evaluation in 17 pediatric patients (12 males, 5 females, age 2-21 years). Single doses of 12 mg/m^2 (N=2), 14 mg/m^2 (N=1), 18 mg/m^2 (N=5) or 20 mg/m^2 (N=9) of NOVANTRONE were given as rapid intravenous infusions over 3-35 minutes. 6 patients were diagnosed as having some hepatic function impairment. Blood samples were collected and analyzed as described in Report #41.

6. Study Results:
(1) Plasma data were best fitted into a 3 compartment model (Table 39.1, $t_{1/2}\alpha$ = 3.6 min, $t_{1/2}\beta$ = 25.2 min, $t_{1/2}\gamma$ = 2.86 hr).

(2) The large volume of distribution (Vdr = 168.1 ± 71.8) indicates that mitoxantrone is probably highly bound to tissue in a "deep" compartment. The small value of K_{31} suggests that the release of mitoxantrone from deep tissue-binding sites is slow.

(3) A t-test comparison of the pharmacokinetic parameters of patients having hepatic impairment with patients having normal liver function showed no significant difference (Table 39.2).

(4) A summary of pharmacokinetic parameters for individual patients is shown in Table 39.3).

(5) Bimodal distribution of Vdss (peaks at 45L/m^2 and 110L/m^2) suggest that two groups of subjects were present in the 17 patients studied. It is observed that those children with largest Vdss also had larger CL_T. The younger children (less than 5 years old) were among those with lower Vdss and CL_T values.

TABLE 39.1

Mean Mitoxantrone Pharmacokinetic Parameters (N=17)

Parameter	Units	Mean*	S.D. %	Min	Max
α	min^{-1}	0.1924	74.6	0.06100	0.55900
β	min^{-1}	0.02744	48.4	0.01220	0.05740
γ	min^{-1}	0.00404	146.6	0.00135	0.00484
$t_{1/2}^{\alpha}$	min	3.60	74.6	1.24	11.36
$t_{1/2}^{\beta}$	min	25.2	48.4	12.08	56.82
$t_{1/2}^{\gamma}$	hr	2.86	146.6	2.39	8.56
k_{12}	min^{-1}	0.0360	93.9	0.00879	0.20434
k_{21}	min^{-1}	0.0430	60.2	0.01652	0.11619
k_{13}	min^{-1}	0.02596	78.4	0.005290	0.11238
k_{31}	min^{-1}	0.00400	46.8	0.001890	0.00900
k_{10}	min^{-1}	0.07427	66.4	0.026740	0.24186
V_1	L/m^2	6.34	114	0.91	26.93
V_2	L/m^2	5.31	82.3	0.74	7.89
V_3	L/m^2	41.2	74.4	13.95	128.17
Vd_{ss}	L/m^2	54.7	76.3	16.60	144.59
Vd_{γ}	L/m^2	168.1	71.8	45.14	586.18
Cl_T	$L/min/m^2$	0.471	59.6	0.183	1.134

* Geometric Mean.

TABLE 39.2

Comparison of the Mean Mitoxantrone Pharmacokinetic Parameters of "Normal" Pediatric Patients with Pediatric Patients Having Hepatic Dysfunction

Parameter		Normal N=11		Hepatic Dysfunction N = 6		R**	95% Confidence Limits of R (%)	
		Mean*	C.V.%	Mean	C.V.%	%	Lower	Upper
α	(min^{-1})	0.1586	62.4	0.1816	71.4	114	81	162
β	(min^{-1})	0.0344	123.2	0.0325	44.3	95	62	143
γ	(min^{-1})	0.00276	39.5	0.00289	37.4	105	101	109
k_{12}	(min^{-1})	0.0375	91.1	0.0430	114.5	115	36	370
k_{21}	(min^{-1})	0.0414	63.5	0.0461	59.0	111	57	216
k_{13}	(min^{-1})	0.0280	68.0	0.0227	100.3	81	30	218
k_{31}	(min^{-1})	0.0042	46.7	0.0037	50.7	89	52	153
k_{10}	(min^{-1})	0.0632	67.8	0.0999	57.3	158	81	309
V_1	(L/m^2)	7.65	112.9	4.50	118.0	59	16	210
V_2	(L/m^2)	6.93	73.1	3.27	81.3	47	20	112
V_3	(L/m^2)	191.2	125.0	40.3	90.1	21	7	66
Vd_{ss}	(L/m^2)	68.5	69.7	36.2	76.1	53	24	119
Vd_{γ}	(L/m^2)	175.5	66.8	155.4	86.5	89	37	213
Cl_T	$(L/min/m^2)$	0.484	60.4	0.449	63.4	93	47	184

* All mean values are geometric means used for comparison.

** R = Hepatic Dysfunction/Normal x 100.

Table 37.3

SUMMARY OF MITOXANTRONE MACROSCOPIC PHARMACOKINETIC PARAMETERS DURING A PHASE I CLINICAL STUDY

Patient	Dose (mg/m^2)	α (min^{-1})	β (min^{-1})	γ (min^{-1})	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)	$t_{1/2\gamma}$ (hours)	Cl ($L/min/m^2$)
F.A.	20	0.0826	0.0343	0.00412	8.39	20.21	2.80	0.772
C.B.	18	0.103	0.0215	0.00187	6.73	32.24	6.18	0.965
B.B.	20	0.559	0.0508	0.00446	1.24	13.64	2.59	0.201
B.G.	18	0.320	0.0574	0.00484	2.17	12.08	2.39	0.906
J.H.	20	0.150	0.0356	0.00307	4.62	19.47	3.76	0.718
H.H.	20	0.213	0.0299	0.00174	3.25	23.18	6.66	0.504
S.J.	18	0.150	0.0199	0.00373	4.62	34.83	3.10	0.510
B.M.	12	0.140	0.0467	0.00135	4.95	14.84	8.55	0.554
J.M.	20	0.483	0.0305	0.00365	1.44	22.73	3.17	0.220
H.M.	20	0.0726	0.0168	0.00292	9.55	41.26	3.96	0.725
G.M.	16	0.199	0.0182	0.00265	3.48	38.09	4.36	0.208
J.P.I	20	0.0610	0.0165	0.00194	11.36	42.01	5.97	1.134
J.P.	18	0.206	0.0530	0.00372	3.36	13.08	3.11	0.515
B.R.	18	0.0839	0.0146	0.00199	8.26	47.48	5.80	0.545
B.S.	20	0.160	0.0241	0.00342	4.33	27.62	3.37	0.321
R.T.	12	0.152	0.0122	0.00199	4.56	56.82	5.81	0.183
H.W.	20	0.313	0.0365	0.00333	2.21	18.99	3.47	0.504

SUMMARY OF MITOXANTRONE MICROSCOPIC PHARMACOKINETIC PARAMETERS DURING A PHASE I CLINICAL STUDY

Patient	Dose (mg/m^2)	k_{12} (min^{-1})	k_{21} (min^{-1})	k_{13} (min^{-1})	k_{31} (min^{-1})	k_{10} (min^{-1})
F.A.	20	0.01050	0.05844	0.01565	0.00672	0.02969
C.B.	18	0.02465	0.03299	0.01254	0.00234	0.05348
B.B.	20	0.20434	0.08741	0.11238	0.00713	0.20306
B.G.	18	0.10480	0.11060	0.06852	0.00900	0.08934
J.H.	20	0.02311	0.04535	0.02988	0.00420	0.08570
H.H.	20	0.01788	0.03322	0.01529	0.00189	0.17667
S.J.	18	0.05036	0.03299	0.02739	0.00591	0.05690
B.M.	12	0.01428	0.11619	0.01758	0.00201	0.03786
J.M.	20	0.14440	0.04489	0.08069	0.00495	0.24186
H.M.	20	0.01177	0.02108	0.02045	0.00495	0.03410
G.M.	14	0.05432	0.02589	0.04073	0.00791	0.09458
J.P.I	20	0.00879	0.02108	0.00529	0.00220	0.04217
J.P.	18	0.05022	0.09644	0.02863	0.00511	0.08243
B.R.	18	0.02883	0.03041	0.01147	0.00380	0.02674
B.S.	20	0.03753	0.03369	0.01765	0.00413	0.09313
R.T.	12	0.03756	0.01652	0.02889	0.00277	0.08039
H.W.	20	0.10493	0.06154	0.05595	0.00494	0.12588

SUMMARY OF MITOXANTRONE VOLUME PHARMACOKINETIC PARAMETERS DURING A PHASE I CLINICAL STUDY

Patient	Dose (mg/m^2)	V_1 (L/m^2)	V_2 (L/m^2)	V_3 (L/m^2)	Vd_{ss} (L/m^2)	Vd_γ (L/m^2)
F.A.	20	26.02	4.68	60.54	91.24	187.44
C.B.	18	17.67	13.20	96.77	125.64	305.28
B.B.	20	8.99	2.32	15.69	18.93	43.14
B.G.	18	11.04	10.46	86.06	105.56	203.83
J.H.	20	8.37	4.27	99.34	72.18	233.80
H.H.	20	1.72	8.93	13.95	16.60	175.43
S.J.	18	8.96	13.67	41.48	64.10	136.63
B.M.	12	14.62	1.88	128.17	144.59	489.58
J.M.	20	8.91	2.93	14.86	18.70	68.48
H.M.	25	21.25	11.86	87.71	120.82	248.35
G.M.	14	2.20	4.63	22.97	29.80	78.67
J.P.I	20	26.93	11.23	64.94	103.09	306.18
J.P.	18	6.24	3.25	34.99	44.48	138.36
B.R.	18	20.20	19.33	78.84	117.76	279.76
B.S.	20	3.45	3.68	10.76	21.81	93.75
R.T.	12	2.27	5.17	23.68	31.12	91.87
H.W.	20	4.03	6.88	43.66	56.57	151.41

7. Deficiencies:
(1) Inaccurate estimation of pharmacokinetic parameters.
Same as Report #41, Deficiencies (1).

(2) Inconclusive results with regard to the effects of hepatic disfunction on the drug's pharmacokinetics.

This study showed that the mean pharmacokinetic parameters obtained from pediatric patients with hepatic disfunction is not significantly different from those obtained from pediatric patients with normal liver function. However, due to (a) inaccurate estimation of these parameters, (b) conflicting information found in adult patients (Report #41 Deficiencies (2)), and (c) no available supportive studies to justify the investigator's results. No conclusion can be made at this time.

8. Conclusion:

Same conclusion as for Report #41 for the blood level data.

D. Report #42

1. Title:
"Pharmacokinetics of Mitoxantrone HCl (CL 232,315) following administration of a single intravenous dose in patients with acute Myelogenous leukemia".

2. Investigators:
Professor J. L. Michaux
Dr. R. Hulhoven
Professor J. M. Defry
Catholic University of Louvain, Brussels, Belgium.

3. Objective:
To assess the pharmacokinetics of mitoxantrone in patients with acute myelogenous leukemia.

4. Formulation:
10 ml of sterile solution contains:

active ingredient	mitoxantrone•2HCl 20 mg (as free base)/ml
inactive ingredients	sodium metabisulfite 0.2% w/v sodium chloride 0.8% w/v

5. Study Design & Procedure:
This study was an open-label clinical efficacy and safety evaluation in 5 patients (3 males, 2 females, age 20-56 years) with acute myelogenous leukemia in relapse or refractory to other therapy. Each patient received a single dose of 24 mg/m^2 as a continuous intravenous infusion over a 30 min period. Blood samples (0-72 hr) were collected. Urine samples (from patients 1 and 2) were collected for 72 hrs.

6. Analytical Procedure:
Plasma samples were stored and analyzed by the method of Ostroy and Gams. Assay sensitivity was 10 ng/ml.

TABLE : 42.1 :

ESTIMATED MITOXANTRONE PHARMACOKINETIC PARAMETERS - DOSE 24 mg/m^2

		SUBJECTS						
PARAMETER	UNITS	1	2	3	4	5	MEAN	(S.D.)
α	Min^{-1}	0.14	0.15	0.13	0.10	0.33	0.17	(0.09)
β	Min^{-1}	0.02	0.076	0.018	0.011	0.048	0.034	(0.03)
γ	Min^{-1}	0.0013	0.00086	0.0020	0.0011	0.0012	0.0013	(0.000)
$t_{\frac{1}{2}\alpha}$	Min	4.95	4.62	5.33	6.93	2.1	-	
$t_{\frac{1}{2}\beta}$	Min	34.7	9.12	38.5	63.0	14.4	-	
$t_{\frac{1}{2}\gamma}$	Hr.	8.9	13.4	5.8	10.5	9.6	-	
K_{12}	Min^{-1}	0.035	0.013	0.018	0.020	0.095	0.036	(0.03)
K_{21}	Min^{-1}	0.029	0.105	0.022	0.014	0.075	0.049	(0.04)
K_{13}	Min^{-1}	0.026	0.052	0.030	0.016	0.067	0.038	(0.02)
K_{31}	Min^{-1}	0.0018	0.0017	0.0028	0.0014	0.0018	0.019	(0.000)
K_{10}	Min^{-1}	0.069	0.057	0.075	0.063	0.14	0.081	(0.03)
V_c	L/M^{-2}	4.26	7.16	4.51	5.98	2.99	4.98	(1.6)
$V_{d\gamma}$	L/M^{-2}	231	475	171	351	356	317	(119)
CL_T	L/M^{-2}	0.29	0.41	0.34	0.38	0.42	0.37	(0.05)

7. Study Results:

(1) The plasma drug data were best fit to a 3 compartment model. (Table 42.1; $t_{1/2}\alpha$ = 4.1 min, $t_{1/2}\beta$ = 19.8 min, $t_{1/2}\gamma$ = 8.9 hr, Vdr = 317 L/m^2, CL_T = 0.37 L/m^2)
(2) Urine recovery in 72 hr was 7.1% for patient #1 and 9.7% for patient #2.

8. Deficiencies:

(1) Inadequate estimation of pharmacokinetic parameters. Due to the insensitivity of the assay method, only those plasma data obtained in 0-12 hr (0-6 hr in one patient and 0-8 hr in another patient) could be used to derive pharmacokinetic parameters. The estimated terminal half-life was 8.9 hr which is 1/4 the values estimated in Report #40 and #37. Furthermore, the urine data obtained from patients #1 and #2 demonstrated that the drug was being excreted after 6-8 half-lives had passed. These observations indicate that the terminal half-life of the drug has been inadequately estimated in this study.

(2) Lack of individual urine data.
(3) Lack of assay valudation data.

This report does not provide assay procedure and assay validation data. Therefore, the pharmacokinetic parameters reported in this study cannot be accurately evaluated.

9. Conclusion:

The Division of Biopharmaceutics finds the pharmacokinetic parameters determined in this study not acceptable.

E. Report #36

1. Title:
"Pharmacology of Mitoxantrone in Cancer Patients" (see attached article)

2. Study Design and Procedure:
11 patients participated in this study. 6 patients who had normal liver and kidney functions were dosed at 1-3 mg/m^2. The remaining 5 patients who had abnormal liver and third space were dosed at 2-12 mg/m^2 (see Report #37 for detailed procedure).

3. Study Results:
The pharmacokinetic parameters for each group of patients are summarized in Table 36.1 and 36.2.

4. Deficiency:
Due to the lack of individual plasma and urine data and assay validation data the pharmacokinetic parameters reported in this study can not be meaningfully evaluated.

5. Conclusion:
The Division of Biopharmacuetics finds Report #36 not acceptable.

F. Report #37

1. Title:
"Clinical Kinetics of 1,4-dihydroxy-5,8 bis [[2-[(2-hydroxyethyl)amino] ethyl] amino]-9, 10-anthracenedione"

See attached article

2. Study Design and Procedure:
Six patients with metastatic cancer (5 males, 1 female, age 37-68 years) received a single dose of 1 mg/m^2 (N=1), 2 mg/m^2 (N=1), or 3 mg/m^2 (N=4) of mitoxantrone as an IV bolus. Blood samples (0-96 hr) were collected. Urine was collected at 6 hr intervals for the first 24 hr and then daily for 72 hr. ^{14}C radioactivity was determined by liquid scintillation counting and mitoxantrone (parent drug) was analyzed by HPLC using unlabelled mitoxantrone as marker.

3. Study Results:
The pharmacokintics parameters and urine data were summarized in Table 37.1.

4. Deficiencies:
Due to the lack of raw data and assay validation data, the parameters reported in this study can not be accurately evaluated.

5. Conclusion:
Same as Report #36, conclusion.

~~Report No. 21~~

Cancer Chemother Pharmacol (1982) 8: 113–117

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Report # 36

Pharmacology of Mitoxantrone in Cancer Patients

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Summary. Radioactive mitoxantrone was administered at doses of 1–12 mg/m² by rapid IV infusion to 11 patients. Of the 11 patients, six had normal liver and kidney function tests while the remaining five had abnormal third space and/or hepatic dysfunction. In the former group, the initial $t_{1/2}$ was 13.7 min and terminal $t_{1/2}$ was 37.4 h. The apparent volume of distribution was 13.8 l/kg. The total clearance rate was 230.7 ml/kg/h. The recovery of unchanged mitoxantrone from urine was 6.8% at 24 h and 7.3% at 72 h, while the corresponding recovery of total radioactivity was 9.4% at 24 h and 11.3% at 72 h. In the five patients with abnormal liver function or third space the initial $t_{1/2}$ was variable and ranged from 11.5–63.6 min, and the terminal $t_{1/2}$ ranged from 53.3–173.2 h, whereas the total clearance rate varied from 52.7–170.2 ml/kg/h. However, the cumulative urinary excretion of unchanged mitoxantrone was similar to that of patients with normal hepatic function: 3.9 at 24 h and 5 at 72 h. Biliary excretion was studied in one of these patients, who had jaundice and hepatic impairment; only 2.3% of ^{14}C was excreted in 24 h and 2.7% in 96 h, of which 39% and 41%, respectively, were unchanged mitoxantrone. Our results suggest that mitoxantrone is taken up rapidly by tissue from which it is released slowly. Reduction of mitoxantrone dose is therefore advisable in patients with liver dysfunction or abnormal third space.

Introduction

Mitoxantrone (NSC-301739), 1,4-dihydroxy-5,8-bis[(2[(2-hydroxyethyl)amino]ethyl)amino]-9,10-anthracenedione dihydrochloride) (Fig. 1), a new anthracenedione derivative, has shown antitumor activity superior or equal to that of doxorubicin in several animal tumor systems, but apparently without cardiotoxicity [4, 7, 8, 11, 12]. Although its mechanism of action remains obscure, preliminary evidence suggests that, like doxorubicin, mitoxantrone intercalates with DNA [2, 3] and is a potent inhibitor of RNA and DNA synthesis of cultured mouse lymphoma L5178Y cells. Compared with doxorubicin on an equimolar basis, mitoxantrone is approximately seven times more potent in inhibiting the incorporation of ^3H-uridine and four times more potent in inhibiting the incorporation of ^3H-thymidine in these cells [1].

In beagle dogs and cynomolgus monkeys, myelosuppression and toxic gastrointestinal manifestations, such as emesis and bloody diarrhea, appeared to be the major dose-limiting toxic effects of mitoxantrone. Other toxic effects included weakness, weight loss, swollen limbs, labored breathing, hyperthermia, excessive salivation, and lacrimation [1]. Because of its impressive anticancer activity in experimental systems and its apparent lack of cardiotoxicity, mitoxantrone has been selected for phase I-II clinical trial.

Von Hoff et al. recently reported the maximum tolerated dose of mitoxantrone to be 14 mg/m² given by IV infusion every 4 weeks [10]. Myelosuppression was the dose-limiting factor, while other toxic effects.

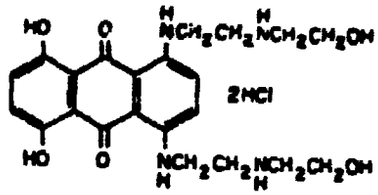

Fig. 1. Structural formula of 1,4-dihydroxy-5-8 bis[(2[(2-hydroxyethyl)amino]ethyl)amino]9,10-anthracenedione dihydrochloride: NSC 301739, mitoxantrone

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such as nausea, vomiting, and diarrhea, were uncommon. There was no evidence of cardiotoxicity, renal failure, or alopecia in the 25 patients studied.

We now describe our clinical pharmacology studies of mitoxantrone in 11 patients with metastatic cancer; the results may be of interest to investigators involved in phase I/II trials of this drug.

Materials and Methods

Mitoxantrone specifically labeled with ^{14}C in all four carbons of the bis-(2-hydroxyethyl) moiety (specific activity 11.2 mCi/mmole, 95% pure chemically and radiochemically) and the unlabeled drug were generously supplied by the Drug Developmental Branch of the National Cancer Institute; they were specifically designated for human use. The drug was formulated in normal saline at a concentration of 50 μg/ml and administered to patients as an IV infusion over 15 min.

Eleven patients who had histologically proven malignancy and had failed conventional chemotherapy were recruited for the study. Informed consent was obtained in all patients before treatment. Two patients had abnormal liver biochemical test (elevation of serum glutamic oxaloacetic transaminase, alkaline phosphatase, and bilirubin to more than twice normal values), two patients had ascites, and one had massive leg edema secondary to lymphatic obstruction from metastatic cancer. All patients had normal renal function tests (normal blood urea nitrogen and serum creatinine) and normal blood cell count (WBC above 3,000/μl and platelet count above 100,000/μl). Patients' characteristics are listed in Table 1.

Sample Collection. Blood (10 ml) was collected via a heparin lock at 0, 15, and 30 min, and at 1, 2, 4, 6, 12, 24, 48, and 72 h. Plasma was separated by centrifugation for determination of mitoxantrone. Urine was also collected, at 6-h intervals for the first 24 h and then daily up to 72 h.

Radiochemical Technique. Radioactivity was determined with a Packard Tricarb liquid scintillation spectrometer model 2650; quenching was corrected by the external standard channels ratio method; for ^{14}C the counting efficiency was about 90%. Plasma or urine (0.2 ml) was counted in 11 ml PCS, a commercial phase-combining counting solution available from Amersham, Arlington Height, IL.

Determination of Unchanged Mitoxantrone by High-pressure Liquid Chromatography. All plasma samples (10 volumes) were deproteinized with 20% sulfosalicylic acid (1 volume) and the supernatant was adjusted to pH 10 with 8 *N* NaOH; urine samples were also made alkaline to pH 10. The deproteinized plasma or urine was extracted with an equal volume of chloroform-isopropanol (1 : 1, v/v), accompanied by vigorous agitation with a vortex mixer for 5 min, and centrifuged at 12,500 *g* (Sorvall RC2-B centrifuge) for 20 min. The organic phase was removed and evaporated to dryness in a sample concentrator (Brinkmann Instrument, Inc., Model SC-42 Westbury, NY, USA) under a stream of nitrogen. The residue was reconstituted with 150 μl distilled water for injection into the high-pressure liquid chromatography (Water Associates Model 204), for which a Waters μ Bondapak C_{18} reverse-phase column (30 cm × 40 mm ID) was used. The elution system consisted of 0.1 *M* acetate buffer, pH 3.95, in 30% methanol, at a flow rate of 2 ml/min under 2,000 psi, with the UV detector set at 254 nm. The location of the unchanged mitoxantrone was ascertained by running unlabeled mitoxantrone through the column. The retention time for mitoxantrone was 16 min. Eluate was collected at 2-min intervals for 20 fractions. The fractions were mixed with 11 ml PCS and the radioactivity was counted in the liquid scintillation counter. The recovery of total radioactivity was greater than 90%.

Computation of the Results. Nonlinear least-square regression analysis of the results was performed with the aid of the PROPHET program. Best fit was obtained on the basis of the conventional open two-compartment model. Pharmacokinetic parameters were calculated in the usual fashion.

Results

The pharmacokinetic parameters of mitoxantrone in the first six patients who had normal liver biochemical tests and no abnormal third space is shown in Table 2. The harmonic mean initial half-life of the drug was 13.7 min and the terminal half-life was 37.4 h. The apparent volume of distribution was about 13.8 l/kg, suggesting extensive tissue binding. The mean total clearance was 238.7 ml/kg/h, twice as high as the creatinine clearance in man.

The cumulative urinary excretion of unchanged drug for the first six patients was 7.3% at 72 h, whereas the corresponding recovery of total radioactivity was 11.3% at 72 h (Fig. 2). Therefore unchanged mitroxantrone accounted for 64.6% of the total radioactivity.

Table 3 shows the pharmacokinetic parameters of the remaining five patients who had abnormal liver biochemical tests and/or abnormal third space. The harmonic mean terminal half-life was 70.7 h, which is significantly longer than that of the first six patients, and the mean clearance was 100.7 ml/kg/h, which is significantly lower than that in the six patients of the first group. The computer-generated plasma clearance curve for total unchanged mitoxantrone in a patient with normal hepatic and renal function (patient 4) in comparison with that of a patient with ascites (patient 7) is shown in Fig. 3.

The mean cumulative urinary excretion of unchanged mitoxantrone in this group of patients was 5.1% at 72 h and the corresponding total ^{14}C was 10.2%, which is not statistically different from that in the first six patients.

Biliary excretion of mitoxantrone was studied in patient 11, who had an indwelling T-tube and presented with jaundice and abnormal liver function tests. The excretion of both total radioactivity and unchanged mitoxantrone in the bile was minimal: only 2.3% of the ^{14}C in 24 h, and 2.7% in 96 h, of which 39% and 41%, respectively, were unchanged drug.

Table 1. Patient diagnosis and characteristics

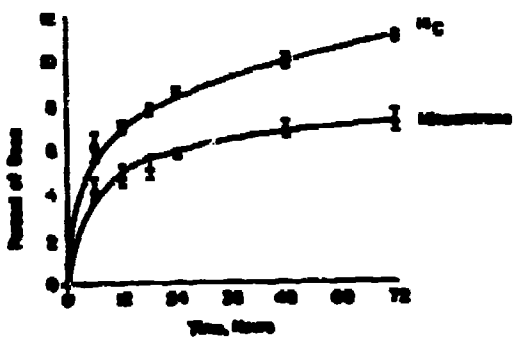
Patient	Age	Sex	DHAQ dose mg/m²	Diagnosis
1	59	M	1	Hepatocellular carcinoma
2	68	M	2	Squamous cell carcinoma of the lung
3	61	M	3	Small cell carcinoma of the lung
4	46	M	3	Adenocystic carcinoma of the epiglottis
5	37	F	3	Squamous cell carcinoma of the neck
6	55	M	3	Malignant melanoma
7	52	M	4	Synovial sarcoma with leg edema
8	60	M	3	Adenocarcinoma of the rectum ascites
9	57	F	3	Epidermoid carcinoma of the lung hepatic impairment and ascites
10	56	F	12	Chronic lymphocytic leukemia, ascites
11	66	M	2	Adenocarcinoma of the colon hepatic impairment

Table 36.1 (patients with normal liver & kidney functions)

Table 2. Mitoxantrone pharmacokinetic parameters

Patient	Half-lives		Vd	Clearance rate	Urinary excretion, % dose			
	Initial	Terminal			Unchanged drug		Total C^{14}	
	(min)	(h)	(l/kg)	(ml/kg/h)	24 h	72 h	24 h	72 h
1	16.9	26.5	7.8	202.9	[illegible]	10.1	10.1	13.6
2	10.2	32.9	5.7	119.6	12.5	n.c.[a]	14.9	n.c.[a]
3	27.0	46.7	22.6	335.2	5.7	5.1	8.3	10.2
4	28.0	41.9	13.8	228.9	7.5	10.5	9.9	14.1
5	7.9	39.1	21.6	383.3	4.0	5.7	6.9	9.1
6	12.2	46.8	11.0	162.5	3.5	5.1	6.2	9.4
Mean ± SE	13.7[b] ± 4.2	37.4[b] ± 4.2	13.8 ± 2.9	238.7 ± 41.5	6.8 ± 1.3	7.3 ± 1.2	9.4 ± 1.3	11.3 ± 1.1

[a] Not collected
[b] Harmonic mean



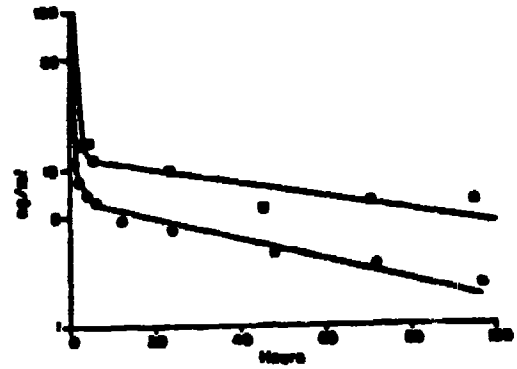


Fig. 2. Cumulative urinary excretion of radioactivity following administration of ^{14}C-mitoxantrone. ●——●, excretion of total radioactivity; ▲——▲, excretion of unchanged mitoxantrone. Values are the means ± SE for six patients

Fig. 3. Plasma disappearance of mitoxantrone postinfusion in patients 4 and 7. The *solid line* is the computer-generated semilogarithmic plot of the non-linear least-square fit of the data; the *closed circles* are the observed values. (■——■, observed values from patient 7; ●——●, observeds values from patient 4; ——) fitted values

Table 36.2 (patients with liver dysfunction & third space)

Table 2. Mitoxantrone pharmacokinetic parameters in patients with cytotoxopathy or third space

Patient	Half-lives		Vd	Clearance rate	Urinary excretion, % dose			
	Initial	Terminal			Unchanged drug		Total C^{14}	
	(min)	(h)	(l/kg)	(ml/kg/h)	24 h	72 h	24 h	72 h
7	25.8	53.3	5.4	70.1	6.5	7.9	9.2	13.9
8	11.5	73.2	15.2	60.8	4.3	5.3	8.2	10.4
9	24.4	69.3	17.0	179.3	2.9	3.4	3.9	5.6
10	69.6	57.7	4.4	52.7	2.5	n.c.[a]	2.7	n.c.[a]
11	37.1	69.3	15.0	149.6	3.3	3.8	9.4	10.8
Mean ± SE	23.9[b] ± 2.2	76.7[b] ± 23.4	11.4 ± 2.7	100.7 ± 24.5	3.9 ± 0.7	5.1 ± 1.0	6.7 ± 1.6	10.2 ± 1.7

[a] Not collected
[b] Harmonic mean

Discussion

The clinical pharmacokinetics of mitoxantrone have certain unusual features. The elimination of the drug from the plasma was relatively slow, with a terminal half-life of about 37 h, compatible with the low urinary excretion of 7% of the administered dose in 72 h (Table 2). The hepatobiliary excretion rate of this agent in man remains largely unknown, although we observed that very little of it was found in the bile of a patient, who, however, had extensive liver disease. Since in the dog the rate of biliary excretion of mitoxantrone was slow [5], if man resembled the dog in this regard, mitoxantrone is expected to be excreted only slowly in the bile of man also. In both species, measurable amounts of mitoxantrone metabolites were detected in the bile as well as in the urine, but the extent of metabolism was limited. In other words, in man, the renal, hepatic, and metabolic clearance rates of mitoxantrone were all low. To reconcile these findings with the comparatively high total clearance of the drug, which was at least twice the creatinine of man, the conclusion appears to be inevitable that elimination from the plasma must be principally by tissue uptake and binding. This is consistent with the exceedingly large apparent volume of distribution of the drug. Moreover, recent results from our laboratories suggest that mitoxantrone was not only highly bound to plasma protein but also taken up quickly and in high concentrations by blood cells [6].

Regarding the hepatobiliary excretion of mitoxantrone, although the rate was probably slow, the extent of excretion by this route could nevertheless be significant. As mentioned above, in our studies with beagle dogs [5], we observed that the excretion of the drug was slow by either the biliary or the urinary route; unfortunately in these experiments we were unable to collect the bile beyond 5 h. However, in one study we recovered 63% of the administered dose, representing mitoxantrone and metabolites, in the feces of a dog in 24 h. Extrapolating this finding to man, we contend that the predominant route of mitoxantrone excretion in man must be fecal. To confirm this, further studies are now in progress.

Comparing mitoxantrone pharmacokinetics in the two groups of patients, the values of apparent volumes of distribution are not significantly different. However, in patients with hepatic impairment or third space, the average total clearance of mitoxantrone was decreased to less than one-half of that in patients with normal liver function, whereas the terminal half-life of the drug was almost doubled. Similar changes have been reported with many drugs in diseased states involving the liver [9], notwithstanding the fact that the effect of diseased liver on pharmacokinetics is far from simple. In the present study, because renal clearance was not significant in mitoxantrone elimination, the reduction of total clearance of this agent in patients with impaired liver function or third space is most probably attributable to either decreased hepatic clearance or impaired intrinsic metabolic clearance, particularly since we have no evidence that in these patients the tissue deposition of mitoxantrone has been changed.

All but one of our patients were given uniformly low doses of mitoxantrone; the exception was patient 10, who received the drug at a dose of 12 mg/m^2. However, we have elected to include the results obtained in this patient in our report, because even at this seemingly high dose no dose-dependent mitoxantrone pharmacokinetics were apparent. In comparison with the remaining four patients of this group, none of the pharmacokinetic parameters of patient 10 deviated widely from those of the others.

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Our studies have amply demonstrated that knowledge of the pharmacokinetics of a drug is of paramount importance in planning its clinical trial. Patients with moderate liver impairment or abnormality of the third space, such as ascites, are at greater risk for mitoxantrone toxicity. Judicious dosage modifications and frequent monitoring of plasma drug concentrations are advised in these patients to avoid serious consequences of prolonged exposure to mitoxantrone, a drug shown by us to localize and persist in the body.

Acknowledgements. This work was supported by NCI Contract CM-87185. The protocol of the study was approved by the Surveillance Committee of the University of Texas System Cancer Center, MD Anderson Hospital and Tumor Institute.

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Report #37

Clinical kinetics of 1,4-dihydroxy-5,8-bis [[2-[(2-hydroxyethyl)amino]ethyl] amino]-9,10-anthracenedione

The clinical kinetics of 1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10 anthracenedione dihydrochloride (DHAQ) are reported. DHAQ, 1 to 3 mg/m², was administered as an intravenous bolus to six patients with metastatic cancer. Plasma clearance of the drug followed a biphasic pattern with a harmonic mean initial half-life ($t_{1/2}$) of 13.7 min and a terminal $t_{1/2}$ of 37.4 hr. Recovery of unchanged drug in the urine was 6.8% at 24 hr and 7.3% at 72 hr, while the corresponding recovery of total radioactivity was 9.4% and 11.3%. Apparent volume of distribution of DHAQ was about 13.8 ± 2.9 l/kg. Total clearance was 236.7 ml/kg/hr, twice the creatinine clearance.

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1,4-Dihydroxy-5,8-bis[[2-[(2-hydroxyethyl) amino]ethyl]amino]-9,10-anthracenedione dihydrochloride (DHAQ), NSC-301739 (Fig. 1) is one of the several aminoanthracenedione derivatives that exhibit a wide spectrum of antitumor activity against experimental leukemia and solid tumors.[1-6] Preliminary evidence suggests that, like doxorubicin, DHAQ intercalates into DNA[2, 3] and is a potent inhibitor of RNA and DNA synthesis of cultured mouse lymphoma L5178Y cells. When compared with doxorubicin on an equimolar basis, however, DHAQ is approximately seven times as potent

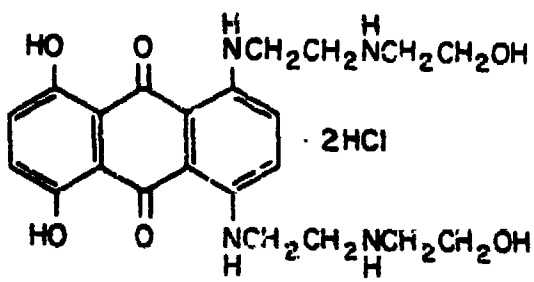

Fig. 1. Structural formula of 1,4-dihydroxy-5,8-bis[[2[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione dihydrochloride.

in inhibiting the incorporation of ^{3}H-uridine and four times as potent in inhibiting the incorporation of ^{3}H-thymidine by L5178Y cells.[1] DHAQ is superior to doxorubicin against L1210 leukemia and has moderate activity against P388 leukemia, which is resistant to doxorubicin. In B16 melanoma and colon carcinoma 26,

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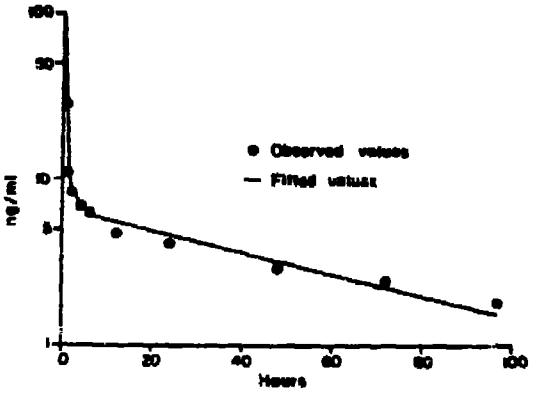

Fig. 2. Plasma disappearance of DHAQ after infusion in patient 4. The solid line is the computer generated semilogarithmic plot of the nonlinear least-square fit of the data and the closed circles are the observed values.

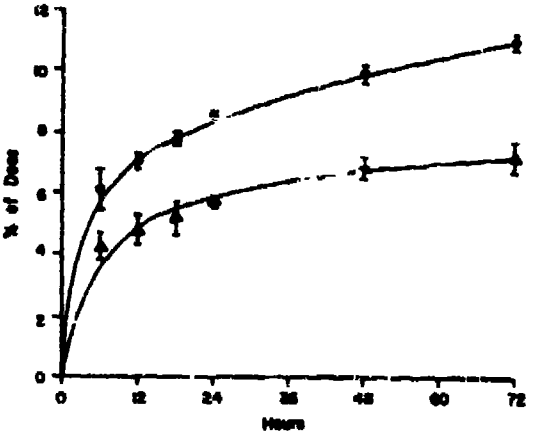

Fig. 3. Total radioactivity (●——●) and unchanged DHAQ (▲——▲). Values are the mean (±SEM) for six patients.

DHAQ has antitumor activity comparable to that of doxorubicin, but it is ineffective against Lewis lung carcinoma and Ridgeway osteogenic sarcoma.[5, 7, 8, 10] Myelosuppression and toxic gastrointestinal manifestations such as emesis and bloody diarrhea appear to be the major dose-limiting toxic effects in beagles and cynomolgus monkeys. Side effects also include weakness, weight loss, swollen limbs, labored breathing, hyperthermia, excessive salivation, and lacrimation.[1] Von Hoff et al.[9] recently reported a phase I study that showed the maximum tolerated dose to be 14 mg/m² and confirmed that myelosuppression is a dose-limiting factor.

Materials and methods

DHAQ, unlabeled and specifically labeled with ^{14}C in all four carbons of the bis(2-hydroxyethyl) moiety, were generously supplied by the Drug Development Branch of the National Cancer Institute. The labeled drug had a specific activity of 11.2 mCi/mmol, with a 95% radiochemical purity by autoradiography. DHAQ was formulated in normal saline solution at a concentration of 0.5 mg/ml for intravenous injection; only freshly constituted solution was used.

Patient selection. All patients had histologically proved malignancy and adequate hepatic function (serum bilirubin, serum glutamic oxaloacetic transaminase, and alkaline phosphatase at the normal level or elevated to less than twice normal) and renal function (normal blood urea nitrogen and serum creatinine). Their leukocyte counts were more than 3000/μl with normal differential counts, and their platelet counts were more than 100,000/μl. Other requirements included complete recovery from the toxicity of previous chemotherapy. Each patient received 100 to 200 mCi, 1 to 3 mg/m² of DHAQ in 12 ml of normal saline solution as an intravenous bolus in 15 min. Pharmacologic studies were performed on day 1 of the first course. Patient characteristics are listed in Table 1.

Sample collection. Blood samples of 10 ml were collected through a heparin lock at 15 and 30 min and 1, 2, 4, 6, 12, 24, 48, 72, and 96 hr after drug. Plasma was separated by centrifugation for determination of total radioactivity and unchanged drug. Urine was also collected at 6-hr intervals for the first 24 hr and then daily for 72 hr.

Radioactivity determination. Plasma or urine samples of 0.2 ml each were mixed with 11 ml of a commercial phase-combining counting solution (PCS) available from Amersham, and the radioactivity was determined in a Packard Tricarb Liquid Scintillation Spectrometer Model 2650 equipped with automatic devices for quenching correction and computation of disintegrations per minute.

Determination of unchanged DHAQ by high-pressure liquid chromatography. All

Table I. Patient characteristics

Patient No.	Age (yr)	Sex	Body weight (kg)	Total dose (mg)	Diagnosis	Prior chemotherapy
1	59	M	60.5	1.8	Hepatocellular carcinoma	5-FU
2	68	M	54.0	3.2	Squamous cell carcinoma of lung	5-FU doxorubicin, mitomycin-C
3	61	M	91.3	6.9	Small cell carcinoma of lung	VP-16, cyclophosphamide, doxorubicin
4	46	M	65.9	4.5	Adenocystic carcinoma of epiglotis	Methotrexate, cisplatin, bleomycin doxorubicin, AMSA
5	37	F	47.5	4.3	Squamous cell carcinoma of neck	Methotrexate, doxorubicin, cytoxan, peptichemio, L-alanosime
6	55	M	66.8	5.4	Malignant melanoma	AMSA, decarbazine

plasma samples (10 vol) were deproteinated with 20% sulfosalicylic acid (1 vol) and the supernatant was adjusted to pH 10 with 8N NaOH; urine samples were also adjusted to pH 10. The deproteinated plasma or urine was extracted with an equal volume of chloroform/isopropanol (1:1, v/v), accompanied by vigorous agitation with a vortex mixer for 5 min, and centrifuged at 48,200 × *g* (Sorvall RC2-B centrifuge) for 20 min. The organic phase was removed and evaporated to dryness in a sample concentrator (Brinkman, Model SC/48) under a stream of nitrogen. The residue was reconstituted with 150 μl of distilled water for injection into the high-pressure liquid chromatography (Water, Model 204), using a Waters μ Bondapak C_{18} reverse phase column (30 cm × 40 mm inner diameter). The elution system consisted of 0.1M acetate buffer (pH 3.96) in 30% methanol at a flow rate of 2 ml/min under 2000 psi and with the ultraviolet detector set at 254 nm. The location of the unchanged DHAQ was ascertained by running unlabeled DHAQ through the column. Retention time for DHAQ was 16 min. Eluate was collected at 2-min intervals for 20 fractions. The fractions were mixed with 11 ml of PCS and radioactivity was counted in the liquid scintillation counter. Recovery of the total radioactivity was more than 90%.

Computation of results. Nonlinear least-square regression analysis of the results was performed with the aid of the PROPHET program. Best fit was obtained based on an open two-compartment model.

Results

We studied six patients: one each took a 1- and 2-mg/m² dose, and four took a 3-mg/m² dose. The computer-generated plasma clearance curve of unchanged DHAQ in patient 4 is shown in Fig. 2 and kinetic parameters of all six patients are shown in Table II. The harmonic mean initial half-life ($t_{1/2}\alpha$) was 13.7 ± 4.2 min and the elimination half-life ($t_{1/2}\beta$) was 37.4 ± 3.7 hr. The apparent volume of distribution was about 13.8 ± 2.9 l/kg. The mean body clearance was 238.7 ± 42.0 ml/kg/hr, twice as high as the creatinine clearance.

The cumulative urinary excretion of unchanged drug was 6.8 ± 1.3% at 24 hr and 7.3 ± 1.2% at 72 hr (Fig. 3). Urinary recovery of total radioactivity was 9.4 ± 1.3% at 24 hr and 11.3 ± 1.1% at 72 hr; unchanged DHAQ therefore accounted for about 64.4% of the total radioactivity at 72 hr.

Hematologic toxicity was minimal in these patients; the median nadir of the leukocyte count was 3000/μl and that of the platelet count was 233,500/μl. Other toxic effects, including nausea, vomiting, fever, and mucositis, were negligible.

Discussion

Like the anthracycline antibiotics, DHAQ also interacts with DNA and induces similar nuclear aberrations and chromosomal damages.[5] DHAQ and its close analogue NSC-287513 are not cell-cycle phase-specific drugs[3, 4, 7] and can kill cells in logarithmic growth or G_0 phase.

Table 37.1

Table II. DHAQ kinetics

Patient No.	t½ α (min)	t½ β (hr)	Vd l/kg	Clearance rate ml/kg/hr	C × t ng/ml × hr	Urinary excretion: Unchanged DHAQ 24 hr	Unchanged DHAQ 72 hr	Total ¹⁴C 24 hr	Total ¹⁴C 72 hr
1	16.9	26.5	7.8	207.9	146.6	7.7	10.1	10.1	13.6
2	10.2	32.9	5.7	119.6	495.5	12.5	NC	14.9	NC
3	27.0	46.7	22.6	335.2	225.5	7.7	5.1	8.3	10.2
4	28.0	41.9	13.8	228.9	298.3	7.5	10.5	9.9	14.1
5	7.9	39.1	21.6	383.3	236.2	4.0	5.7	6.9	9.1
6	12.2	46.8	11.0	162.5	497.5	3.5	5.1	6.2	9.4
Mean	13.7	37.4	13.8	238.7		6.8	7.3	9.4	11.3
± SEM	±4.2*	±3.7*	±2.9	±42.0		±1.3	±1.2	±1.3	±1.1

Vd = volume of distribution; C × t = concentration × time; NC = not collected.
*Harmonic mean.

These properties make it promising for solid tumors with low growth fractions.

Our studies of DHAQ have disclosed several kinetic characteristics of DHAQ of considerable relevance to its phase I-II clinical trial. DHAQ is cleared from the plasma essentially biphasically with a long t½ (37.4 hr) during the terminal phase. This is compatible with its low urinary excretion. In beagle dogs, DHAQ also has a long plasma t½β and is likewise excreted in small amount in the urine.* Since it is not excreted significantly in dog bile, we infer that its heparobiliary excretion in man is probably low; direct evidence is lacking because bile was not available from our six patients. The low biliary excretion of DHAQ in man was established in at least one patient not included in our study. In this patient, extensive liver disease was diagnosed, however. DHAQ is metabolized to some extent in both man and dogs; both the urinary and biliary excretion of the metabolites appears to be low. All considered, it seems paradoxical that the total clearance of DHAQ is relatively high, at least twice that of creatinine. Sequestration in one or several body compartments from which the drug is gradually released must therefore be principally responsible for the rapid clearance of DHAQ. This contention is supported from two other observations: first, the apparent volume of distribution of DHAQ suggests its ready penetration into tissues, and second, the drug is not only highly bound to plasma protein, but also concentratively taken up by nucleated blood cells.* Consequently, persistence of DHAQ with its associated risks must be borne in mind in planning clinical drug trials.

*Lu K. et al: Manuscript in preparation.

*Savaraj N. et al: Manuscript in preparation.

We wish to thank Dr. L. M. Allen for assistance in PROPHET computer analysis.

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G. Report #44

1. Title:
"Clinical Pharmacokinetics of NOVANTRONE Interim Report (RIA)"

2. Investigators and Protocols:
G. Ehninger, M.D. University of Tubingen Medical School, Tubinger, West Germany, Protocol #D3P504T1

R. Gams, M.D. University of Alabama Medical Center, Birmingham, AL, Protocol #D3P44T8

I.C. Henderson, M.D. Dana Farbar Institute, Boston, MA Protocol #D3P72T0

3. Objective:
To assess the pharmacokinetics of NOVANTRONE with special emphasis on the elimination half-life using a radioimmunoassay.

4. Study Design and Procedure:
These were multi-site open-label clinical efficacy studies of mitoxantrone in acute myelocytic leukemia or breast cancer patients (2 males, 5 females, age 46-65 years). The regimens used varied from a single dose of 14 mg/m^2 given every 3 weeks to a daily dose of 7.5 mg/m^2 or 12 mg/m^2 given for 5 days. Blood samples were collected.

5. Analytical method:
RIA was employed to assay the serum concentration of mitoxantrone in blood samples taken at 2 to 23 days after dosing. The assay sensitivity is 75 pg/ml. Metabolite A (dicarboxylic acid, main metabolite in human and animals), at concentration of 1-10 ng/ml serum, has a cross-reactivity of $<1\%$.

6. Study Results:

Serum concentrations and the estimated terminal half-lives of the individual patients are shown in Table 44.1. All of the $t_{1/2}$ values agree (range 10.2-18.4 days) except that of patient 2013 ($t_{1/2}$=40.7 days) in which sampling was performed only on days 2, 3, and 7. The reliability of this value is questionable due to the short period of sample collection. Mean ($\pm$ S.D.) of the remaining 6 $t_{1/2}$ values was 12.4 $\pm$ 3.1 days.

7. Comment:
Only the Henderson patient (M. Dakton) had a sufficient number of data points to obtain a statistically significant correlation of LnCp versus time; the remaining data sets had good correlations but lacked a sufficient number of points to give statistically reliable estimates of $t_{1/2}$.

8. Conclusion:
The Division of Biopharmaceutics finds Report #44 acceptable.

Table 44.1

Serum Concentrations of Mitoxantrone

(Mean S.D. of Three Replicate Analysis by RIA)
ng/mL

Patient No.	Day		$t_{1/2}$ (days)
Study I, Ehninger			
I	8	1.04 ± 0.05	12.8 *
	16	0.48 ± 0.01	
	23	0.47 ± 0.01	
II	8	0.74 ± 0.03	10.4 *
	15	0.26 ± 0.01	
	22	0.29 ± 0.01	
III	9	1.26 ± 0.03	11.7 *
	16	0.62 ± 0.03	
	23	0.55 ± 0.02	
IV	8	0.75 ± 0.01	10.2 *
	15	0.32 ± 0.05	
	22	0.29 ± 0.04	
Study II, Henderson			
Monique Dakton	3	2.07 ± 0.08	10.8 *
6 Pts	5	1.70 ± 0.11	
	8	1.13 ± 0.06	
	11	0.86 ± 0.07	
	16	0.77 ± 0.03	
	21	0.61 ± 0.0	
Study III, Gams			
Patient 2013 Bobby Moore	2	2.58 ± 0.09	40.7
	3	2.23 ± 0.12	
	7	2.27 ± 0.04	
Patient 2015 Norman Bryant	1	2.24 ± 0.15	18.4 *
	2	1.93 ± 0.09	
	3	2.05 ± 0.06	
	7	3.72 ± 0.20	
	21	1.00 ± 0.02	

Mean of * = 12.4 ± 3.1 d

H. Report #54

1. Title:
"CL 232,315 (mitoxantrone), The Isolation, Purification, and Identification of Two Urinary Metabolites Following Intravenous Administration of CL 232,315 in Man."

2. Investigators:
F.S. Chiccarelli et. al.
Lederle Lab.
Pearl River, NY

3. Objective:
To study the mitoxantrone metabolic pathway in man.

4. Analytical Method:
Urine samples from 10 patients were absorbed on glass wool and sep-pack cartridges followed by preparative HPLC. The isolated metabolites were then subsequently identified by MS with a synthetic marker.

TLC was performed using Brinkman glass plates (5602)

5. Study Results:
- Two of the patients collected urine specimens were found to contain large amounts of polar materials. Two metabolites were isolated and purified. The structures are shown in Fig 54.1. The major metabolite A was identified as dicarboxylic acid and the minor metabolite B was identified as monocarboxylic acid.

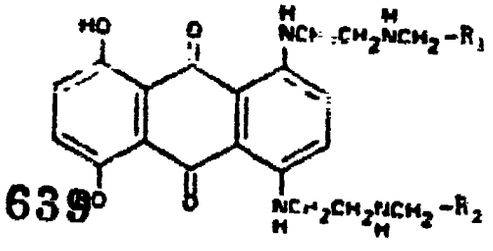
- The elution profile of the 0-24 hr urine sample from one patient contained a high proportion of the more polar metabolite A (Fig 54.2). The total 0-24 hr urine collection (28% of the 12 mg/m^2 IV dose) contained 3.6 mg of metabolite A and 1.8 mg of mitoxantrone.

6. Comments:
This study indicates that the renal excretory pathway in some patients is a significant pathway for the elimination of drug related materials.

7. Conclusion:
The Division of Biopharmaceutics finds Report #54 acceptable

FIGURE 54.1

MOLECULAR STRUCTURE OF MITOXANTRONE AND TWO METABOLITES



R1	R2	MW	Compound
- CH_2OH	- CH_2OH	444	Mitoxantrone
- COOH	- COOH	472	CL 283,918x (Metabolite A^4)
- COOH	- CH_2OH	458	Metabolite B^4

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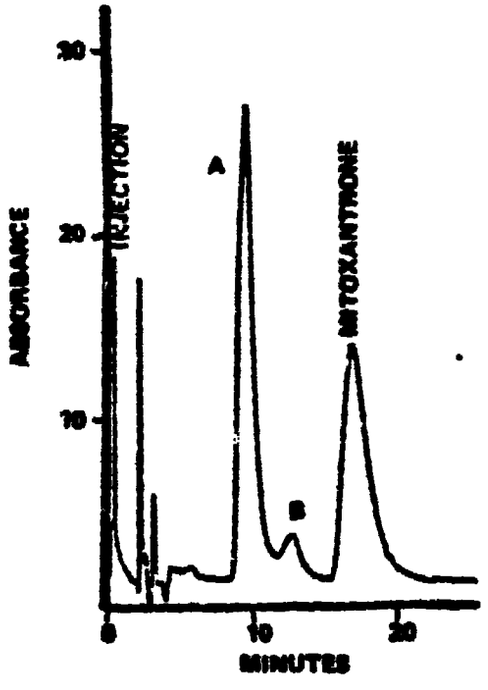
Figure 54.2

HPLC Elution Profile at 658 nm of the 0-24 Hour Urine from Patient D.W. After an Intravenous Dose of Mitoxantrone at 12 mg/m^2



I. Report #58

1. Title:
"Protein Binding of Mitoxantrone"

2. Investigator:
A.P. Tonelli
Lederle Laboratories
Pearl River, NY

3. Objective:
To assess protein binding characteristics of mitoxantrone.

4. Analytical Method:
Equilibrium Dialysis

5. Study Results:
- Mitoxantrone (concentration range 25-455 ng/ml) bound to pooled human plasma had a mean value of 78.3 ± 4.4%.

- The drug (50-500 ng/ml) bound to (a) human serum albumin had a mean value of 75.7 ± 5.5% (SD), and (b) to α_1- acid glycoprotein had a mean value of 66.1 ± 5.9 % (SD)

The extent of binding was independent of drug concentration and there was no evidence of a saturation of binding sites at the concentrations studied.

6. Conclusion:
The Division of Biopharmaceutics finds Report #58 acceptable.

J. Report #59

1. Title:
"In vitro plasma protein binding interactions between ^{14}C CL 232,315 and diphenylhydantoin, doxorubicin, methotrexate, prednisone, prednisolone, heparin and acetylsalicylic acid in human plasma."

2. Investigators:
M. Kohlbrenner
R.A. Bruggman
Lederle Lab.
Pearl River, NY

3. Objective:
To investigate the effect of concomitantly administered drugs on the plasma protein binding of mitoxantrone.

4. Analytical Method:
An equilibrium dialysis method was used with 50 ng/ml and 200 ng/ml of ^{14}C mitoxantrone (therapeutic range in humans) and other test drugs (diphenylhydantoin 20 mcg/ml, doxorubicin 1 mcg/ml, methotrexate 1 mcg/ml, prednisone 200 ng/ml, prednisolone 200 ng/ml, heparin 25 mcg/ml, acetylsalicylic acid 300 mcg/ml).

5. Study Results:
Mitoxantrone Protein Binding
Equilibrium was attained at 7-8 hr.
Optimal mitoxantrone binding was seen at 6-9 hrs.
No difference in binding was observed between 50 ng/ml (76.5 $\pm$ 2.7% bound, n=39) and 200 ng/ml (76.3 $\pm$ 3.6% bound, n=34).
Mean = 76.4 $\pm$ 3.1% bound (n=73). These results suggest that there is no saturation of binding site(s) on the protein within the range of 50-200 ng/ml.

Effects of test drugs on mitoxantrone protein binding.
Table 59.1 shows the equilibrium of ^{14}C-Mitoxantrone in the presence of all added drugs. Table 59.2 shows no statistically significant alterations in mitoxantrone plasma protein binding. Doxorubicin produced some interaction with mitoxantrone but failed to show any significance by two-tailed student t-test.

6. Conclusion:
The Division of Biopharmaceutics finds Report #59 acceptable.

Table 59.1

^{14}C-CL 232,315 Equilibration in the Presence of Added Drugs. Percentage of ^{14}C-CL 232,315 Recovered from Each Control Cell Compartment Post-Dialysis (n=2)

Added Drug	Mitoxantrone Alone		Concomitant Administration	
	Buffer	Spiked Buffer	Buffer	Spiked Buffer
Diphenylhydantoin	48.4	51.6	48.4	51.6
Doxorubicin	48.8	51.2	40.2	59.8
Methotrexate	48.6	51.4	47.7	52.3
Prednisone	47.6	52.4	47.7	52.3
Prednisolone	48.7	51.3	47.4	52.6
Heparin	48.9	51.1	47.9	52.1
Acetysalicylic Acid	49.2	50.8	46.0	54.0

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Table III 59.2

Effects of Drugs on ^{14}C-CL 232,315 Protein Binding

% Unbound CL 232,315 Mean (±SD) [N=4]

Added Drug	Concentration ng/mL	CL 232,315 Concentration ng/mL	% Unbound Without Added Drug	% Unbound With Added Drug	t Statistic	Level of Significance (Two-tailed Test)
Diphenylhydantoin	20.0	50.0	25.2(±2.0)	24.9(±1.1)	0.25	NS[a]
		200.0	24.9(±1.1)	27.3(±2.8)	1.57	NS
Doxorubicin	1.0	50.0	25.3(±1.5)	26.8(±2.2)	1.18	NS
		200.0	27.7(±0.5)[b]	26.2(±1.4)	1.69	NS
Prednisone	0.2	50.0	21.0(±0.7)[b]	20.3(±2.4)	1.21	NS
		200.0	18.9(±0.8)[b]	17.0(±1.5)	1.11	NS
Prednisolone	0.2	50.0	20.7(±1.5)[b]	18.7(±0.6)	2.49	NS
		200.0	21.4(±1.5)[b]	18.1(±0.5)	1.96	NS
Heparin	25.0	50.0	26.5(±5.8)[b]	21.4(±0.8)	1.89	NS
		200.0	20.2(±4.6)[b]	22.4(±0.8)[b]	2.19	NS
ASA	100.0	50.0	27.1(±2.0)[b]	20.9(±1.6)	2.84	NS
		200.0	27.9(±1.0)[b]	20.3(±1.4)	2.96	NS
MTX	1.0	50.0	23.5(±2.1)	22.7(±4.8)	0.36	NS
		200.0	25.4(±3.3)	23.4(±4.5)	0.69	NS
ETOH	5.0	50.0	20.2(±2.3)	18.1(±0.8)	2.01	NS
		200.0	19.9(±1.8)	20.4(±2.2)	0.66	NS

[a] Not significant at p<0.05 level
[b] N=3

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K. Report #33

1. Title:
"Pharmacokinetics and Tissue Distribution of Intravenously Administered ^{14}C-Mitoxantrone in the Rat."

2. Investigators:
S.R. Gautam, et. al.
Lederle Lab.
Pearl River, NY

3. Objective:
To compare the pharmacokinetic properties and tissue distribution of ^{14}C-Mitoxantrone at different iv doses (0.25, 0.5 and 0.75 mg/kg) in the rat.

4. Study Design and Procedure:
36 rats were dosed at 0.25 mg/kg, 42 rats were dosed at 0.5 mg/kg and 36 rats were dosed at 0.75 mg/kg.

Plasma were collected from 5 to 120 days. Urine and feces were collected over 24 hr for the first 10 days, after which the samples were collected cumulatively over a 7 day period until 120 days. Tissue samples were air-dried before combustion in a sample oxidizer.

5. Study Results:
Following iv administration of ^{14}C-Mitoxantrone the total radioactivity had linear, sex-independent and dose-independent pharmacokinetic properties in the rat. Figure 33.1 shows the time course of the total radioactivity in various tissues, blood and plasma at 0.25 mg/kg dose level. The results indicated the following:

1. The distribution of the total radioactivity was extensive.

2. The tissue concentration-time profiles of the total radioactivity showed a much slower rate of decline ($t_{1/2\beta}$ = 20-25 days) compared to that in plasma ($t_{1/2\beta}$ = 11.8 days). Redistribution of the total radioactivity into the cellular compartment may be occurring while the overall tissue concentration was falling. This would explain the slower rate of decline.

3. The persistence of the total radioactivity in the tissues and organs was remarkable with significant amounts detectable through 120 days after a single intravenous dose.

Average tissue concentrations of radioactivity were summarized in Table 33.1-33.3.

6. Conclusion:
The Division of Biopharmaceutics finds Report #33 acceptable.

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Figure 33.1

A Representative Plot Showing Time Course of Total Radioactivity in Plasma, Blood and Various Tissues Following a Single Intravenous Dose of 0.25 mg/kg of ^{14}C-CL 232,315 in Adult Male Rats

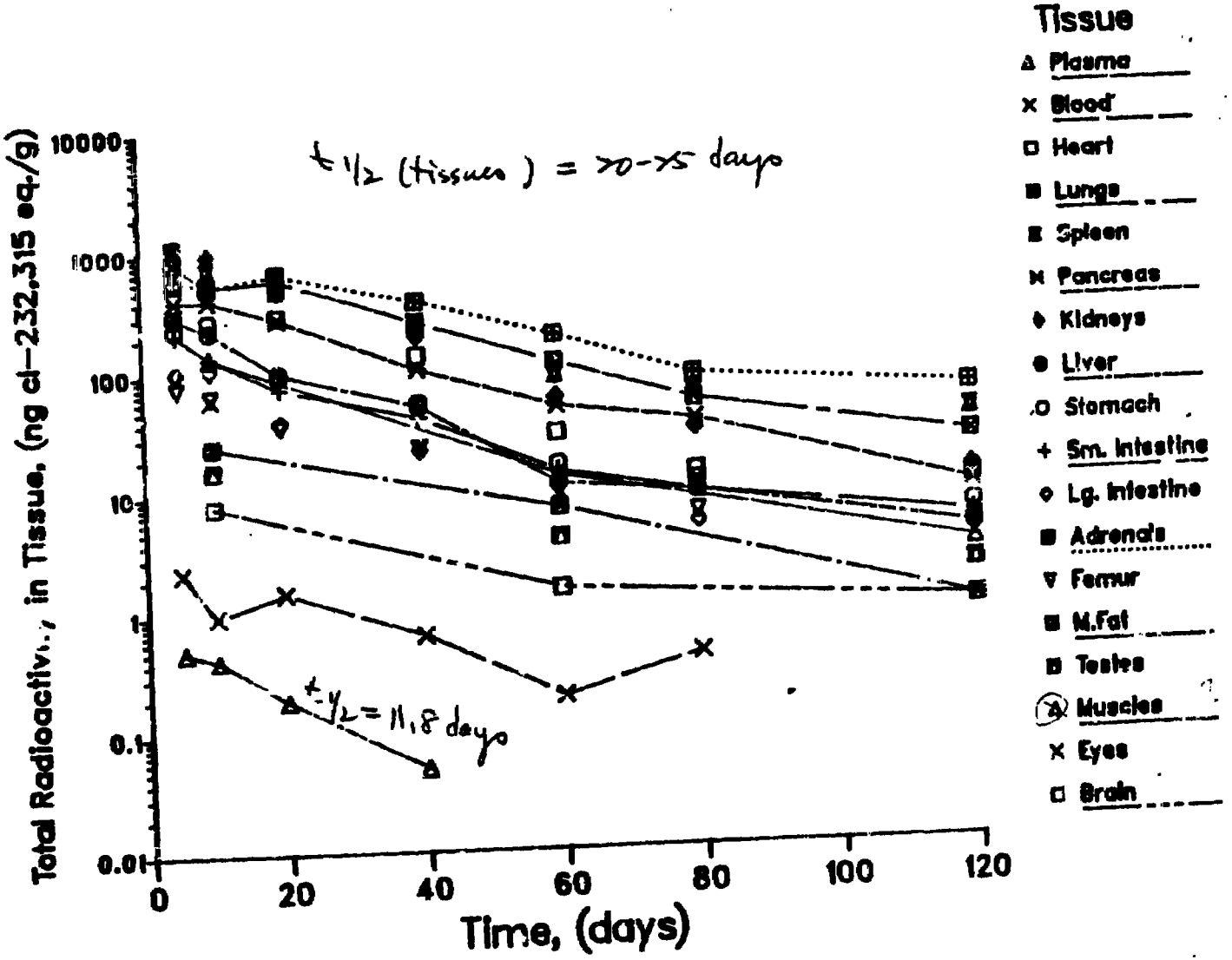

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Table 33,1

Average Tissue Concentrations of Radioactivity in Rats Following a Single Intravenous Dose of 0.25 mg/kg of ^{14}C-CL 232,315 (SN: A8106)

Tissue Concentration (ng CL 232,315 eq./g) (± S.D.)

	Time (Days)											
	5	10		20	40		60		80		120	
Tissue	M	M	F	M	M	F	M	F	M	F	M	F
Blood	1.30 (1.6)	0.98 (0.30)	0.90 (0.26)	1.34 (0.15)	0.64 (0.19)	0.83 (0.32)	0.19 (0.06)	0.42 (0.13)	(0.41 (0.29)	0.35 (0.21)	0 0	0 0
Heart	585.70 (175.0)	557.00 (200.0)	635.30 (61.3)	293.00 (93.4)	134.0 (35.7)	287.3 (42.0)*	29.3 (10.6)	130.0 (54.5)*	13.0 (5.4)	60.3 (32.6)	10.3 (4.0)	14.0 (4.6)
Lungs	841.00 (55.1)	541.33 (80.9)	571.20 (77.4)	590.70 (164.7)	252.33 (24.5)	204.7 (61.5)	110.70 (34.9)	114.3 (15.4)	51.50 (7.4)	81.70 (20.0)	43.30 (7.8)	35.0 (10.5)
Spleen	1236.0 (37.0)	741.33 (206.0)	806.0 (166.5)	480.70 (51.4)	237.0 (40.0)	351.5 (123.5)	87.00 (6.1)	169.0 (59.5)	60.0 (9.0)	196.33 (82.5)	36.00 (26.0)	46.00 (13.8)
Pancreas	409.7 (28.0)	404.7 (135.5)	515.0 (26.9)	280.83 (65.7)	102.33 (43.4)	161.33 (48.2)	42.33 (36.7)	114.0 (30.6)	35.30 (16.4)	62.0 (3.0)	8.70 (2.5)	22.70 (9.5)
Kidneys	1149.50 (305.0)	977.00 (228.5)	1288.33 (360.4)	475.20 (80.0)	192.00 (49.0)	372.00 (134.0)	57.30 (22.0)	167.0 (13.0)*	29.00 (5.0)	130.00 (34.3)*	12.50 (4.8)	22.33 (8.3)
Liver	302.00 (69.3)	227.83 (63.4)	195.83 (56.5)	99.50 (23.4)	50.83 (24.0)	62.83 (15.3)	10.70 (1.5)	28.20 (8.2)*	9.20 (8.1)	16.83 (11.5)	4.33 (1.5)	4.83 (0.7)
Stomach	241.70 (118.5)	276.70 (79.4)	20..5 (25.0)	96.33 (31.2)	53.70 (3.8)	82.5 (16.6)*	15.83 (5.5)	44.30 (8.6)*	11.0 (0.0)	24.33 (5.5)*	6.0 (1.0)	13.33 (3.0)*
Sm. Intes.	217.70 (100.8)	137.00 (42.3)	152.33 (34.7)	74.50 (8.0)	41.33 (11.0)	57.70 (20.0)	14.9 (5.3)	20.2 (5.0)	8.70 (2.10)	31.70 (15.1)	4.70 (0.6)	7.0 (0.0)*
Lg. Intes.	106.5 (5.8)	110.00 (47.6)	77.83 (33.2)	39.20 (8.8)	22.20 (6.5)	26.00 (9.0)	8.00 (1.0)	13.83 (4.5)	5.00 (2.6	9.00 (4.6)	2.20 (0.3)	3.33 (0.6)
Adrenals	634.8 (219.0)	561.0 (301.0)	571.0 (215.0)	662.0 (279.5)	377.0 (101.5)	611.0 (153.0)	187.0 (73.0)	426.0 (89.0)*	83.0 (33.0)	379.0 (143.0)*	58.0 (2.0)	115.0 (44.0)
Femur	77.0 (13.0)	66.0 (3.2)	60.0 (18.0)	34.0 (5.1)	2..0 (3.2)	19.0 (4.7)	10.0 (1.0)	34.2 (33.7)	6.0 (0.6)	8.3 (4.2)	4.0 (1.1)	3.3 (0.6)
Mesenteric Fat		24.70 (14.0)	29.20 (10.9)				4.75 (4.2)	3.33 (1.5)			1.33 (0.6)	3.50 (2.2)
Testes	-	16.33 (2.3)	-	-	-	-	3.83 (0.3)	-	-	-	2.33 (0.6)	-
Ovary	-	-	71.0 (-)	-	-	-	-	48.7 (16.6)	-	-	-	75.7 (3.3)
Muscle -	-	137.00 (50.2)	107.0 (26.9)	-	-	-	13.20 (3.0)	30.0 (10.0)	-	-	2.70 (1.1)	5.3 (0.3)*
Eyes	-	62.00 (22.8)	53.0 (3.2)	-	-	-	13.0 (1.5)	17.2 (4.2)	-	-	5.3 (1.1)	4.3 (2.3)
Brain	-	8.30 (3.2)	6.70 (2.1)	-	-	-	1.50 (0.50)	2.0 (0.0)	-	-	1.0 (0.0)	1.0 (0.0)
Brown Fat	-	-	-	-	-	-	96.0 (18.5)	42.0 (20.0)*	-	-	41.0 (5.0)	34.0 (11.0)

* Statistically significant difference at $p < 0.05$

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Table 93.2

Average Tissue Concentrations of Radioactivity in Rats Following a Single Intravenous Bolus Dose of 0.5 mg/kg of ^{14}C-CL 232,315 (SN: A8187)

Tissue Concentration (ng CL 232,315 eq./g) (± S.D.)

	Time (Days)											
	5	10		20	40		80		80		120	
Tissue	M	M	F	M	M	F	M	F	M	F	M	F
Blood	4.0 (1.4)	2.7 (1.7)	1.9 (0.6)	2.1 (0.9)	0.75 (0.3)	1.55 (0.1)	0.47 (0.1)	0.82 (0.4)	0.23 (0.1)	0.4 (0.1)	0	0
Heart	1143.0 (255.1)	1047.2 (202.6)	1179.7 (225.0)	482.0 (52.0)	222.7 (107.2)	431.0 (93.7)	82.0 (19.0)	76.3 (136.5)	29.7 (0.6)	124.2 (18.3)*	10.7 (3.0)	48.7 (13.0)*
Lungs	1575.7 (461.5)	1558.7 (547.9)	1228.2 (203.4)	1060.0 (207.7)	510.7 (111.6)	493.5 (157.6)	228.3 (53.2)	291.7 (19.3)	152.0 (11.5)	166.90 (26.9)	6.3 (2.5)	7.5 (1.6)
Spleen	1578.7 (223.6)	1665.0 (247.9)	2549.3 (237.7)*	821.5 (79.1)	427.3 (93.5)	459.0 (77.8)	217.3 (5.3)	282.7 (56.2)	106.2 (16.6)	188.3 (15.3)*	70.0 (23.4)	112.5 (34.2)
Pancreas	773.0 (475.3)	921.7 (403.2)	1326.8 (133.7)	427.2 (77.7)	250.7 (74.8)	349.7 (87.7)	154.2 (17.0)	151.0 (49.5)	58.7 (21.6)	109.7 (20.2)*	20.3 (9.0)	112.0 (16.7)*
Kidneys	1594.2 (87.3)	1917.0 (561.0)	3713.8 (1073.4)	815.3 (120.9)	324.0 (24.0)	616.3 (70.2)*	148.0 (56.7)	304.3 (102.9)	105.7 (14.7)	182.5 (29.1)*	22.3 (3.8)	79.3 (4.7)*
Liver	625.0 (132.9)	404.0 (88.7)	674.7 (121.7)	187.0 (39.5)	76.0 (4.6)	91.2 (12.3)	29.2 (12.7)	47.2 (12.5)	13.8 (4.7)	25.3 (3.0)*	29.3 (13.0)	11.7 (4.0)
Stomach	399.3 (131.2)	455.0 (117.7)	574.0 (23.6)	224.3 (13.2)	94.5 (15.9)	162.7 (25.0)*	50.7 (2.5)	68.3 (19.5)	25.7 (4.9)	52.5 (11.3)*	9.0 (3.0)	36.3 (12.8)*
Sm. Intes.	536.8 (195.2)	259.3 (75.6)	548.7 (98.2)*	106.7 (25.5)	67.5 (17.0)	97.3 (8.5)	33.2 (18.9)	45.8 (10.2)	14.3 (2.3)	26.0 (8.9)	10.3 (3.2)	18.7 (3.2)*
Lg. Intes.	226.2 (23.7)	154.8 (27.3)	244.0 (24.3)*	76.3 (9.2)	32.7 (10.0)	45.0 (2.6)	17.8 (5.4)	28.7 (4.2)	12.0 (3.6)	14.0 (1.7)	4.7 (1.1)	9.8 (2.0)*
Adrenals	861.8 (198.4)	721.5 (216.7)	1386.7 (150.0)	849.7 (99.5)	313.3 (35.1)	843.7 (298.9)	384.3 (102.7)	603.8 (191.5)	347.0 (120.6)	603.8 (191.5)*	154.0 (47.8)	287.7 (66.8)
Femur	170.0 (54.6)	115.0 (37.8)	122.7 (19.0)	92.3 (11.1)	37.7 (2.5	31.5 (7.8)	24.8 (1.4)	23.7 (1.1)	13.0 (.0)	13.3 (1.5)	5.3 (1.1)	8.5 (3.9)*
Mesenteric Fat	-	12.5 (10.6)	63.7 (28.9)	-	-	-	31.7 (11.6)	15.5 (2.3)	-	-	3.2 (1.7)	4.0 (2.0)
Testes	-	30.3 (16.6)	- -	-	-	-	7.3 (1.5)	-	-	-	3.8 (0.1)	-
Ovary	-	-	478.0 (229.1)	-	-	-	-	187.7 (43.7)	-	-	-	46.3 (19.1)
Muscles	-	320.3 (52.1)	364.5 (51.3)	-	-	-	22.5 (5.5)	53.5 (9.8)*			4.0 (2.0)	11.7 (1.5)*
Eyes	-	119.7 (26.0)	175.3 (20.2)*				23.2 (2.5)	27.5 (0.5)			7.0 (0)	11.7 (1.1)
Brain	-	11.7 (2.9)	17.5 (5.4)	-	-	-	3.0 (3.0)	4.3 (0.6)	-	-	1.7 (0.6)	2.0 (0.0)
Brown Fat	- -	-	-	-	-	-	140.3 (66.5)	266.0 (128.3)	-	-	92.0 (45.0)	119.0 (52.0)

* Statistically significant difference at $p < 0.05$

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Table 33.3

Average Tissue Concentrations of Radioactivity in Rats Following a Single Intravenous Bolus Dose of 0.75 mg/kg of ^{14}C-CL 232,315
(SN: A8188)

Tissue Concentration (mg CL-232,315 eq./g) (± S.D.)

Tissue	Time (Days) 5 M	10 M	10 F	20 M	40 M	40 F	60 M	60 F	80 M	80 F	120 M	120 F
Blood	6.2 (4.2)	2.4 (0.8)	2.0 (0.5)	3.5 (1.8)	1.9 (1.2)	2.5 (0.8)	0.45 (0.2)	0.62 (0.1)	0.65 (0.2)	1.1 * (0.1)	0.28 (0.2)	0.13 (0.1)
Heart	2136.7 (555.7)	150.2 (250.8)	2536.7 * (546.4)	794.2 (297.3)	573.3 (89.0)	584.7 (56.1)	79.7 (44.7)	302.7 (149.2)	50.0 (11.3)	127.7 (61.0)	18.3 (4.0)	39.2 (21.2)
Lungs	3444.0 (752.0)	1115.2 (457.9)	3619.0 * (866.7)	1398.2 (481.7)	1368.0 (464.3)	644.3 (102.3)	420.0 (294.8)	679.8 (46.1)	296.3 (142.7)	361.3 (111.6)	74.3 (19.3)	101.7 (37.4)
Spleen	3481.7 (889.9)	1682.0 (164.0)	3092.0 * (755.4)	1249.3 (124.8)	902.0 (21.4)	768.0 (138.8)	324.8 (105.8)	635.8 (234.1)	152.7 (53.0)	354.3 (118.2)	80.0 (8.7)	169.7 * (46.7)
Pedarons	2532.3 (418.7)	1020.0 (214.0)	2183.5 (690.8)	736.7 (97.0)	369.0 (144.2)	422.0 * (38.9)	179.0 (54.7)	229.7 (66.6)	113.7 (65.9)	205.0 (61.5)	42.3 (9.4)	122.5 * (5.1)
Kidneys	1902.5 (1418.5)	3572.2 (348.4)	3886.0 (471.2)	1087.0 (27.8)	684.2 (144.8)	1025.0 (175.5)	142.0 (26.8)	436.0 (272.1)	118.3 (1.5)	287.0 * (63.2)	35.2 (4.5)	91.0 * (22.9)
Liver	828.7 (67.4)	835.7 (318.9)	448.0 (166.0)	255.7 (48.0)	102.8 (37.8)	211.3 * (28.9)	30.0 (10.1)	84.3 (25.9)	20.7 (3.0)	31.5 * (1.8)	9.0 (3.5)	12.7 (3.3)
Stomach	875.3 (115.7)	591.3 (84.7)	837.7 (293.4)	296.3 (40.8)	160.3 (84.6)	212.0 (79.6)	74.2 (10.2)	125.3 (34.1)	32.0 (2.0)	64.7 * (8.7)	17.2 (3.6)	25.7 (6.0)
Sm Intem.	337.7 (113.5)	387.3 (355.1)	543.3 (349.0)	192.0 (52.5)	107.7 (22.9)	126.3 (2.1)	34.7 (2.5)	83.7 * (28.7)	19.8 (5.3)	27.3 (11.0)	12.3 (4.5)	20.0 (2.6)
Lg. Intem.	338.3 (72.6)	220.7 (48.0)	360.0 (120.2)	124.3 (21.4)	60.5 (5.8)	63.0 (13.2)	22.2 (3.0)	42.7 * (9.9)	17.0 (6.0)	20.7 (4.9)	7.7 (2.1)	14.0 (3.6)
Adrenals	1563.3 (285.7)	899.7 (653.5)	1485.7 (443.5)	1451.7 (394.6)	970.3 (413.5)	1379.5 (304.5)	478.3 (229.2)	1316.3 * (153.1)	287.3 (39.6)	788.0 (311.4)	198.3 (39.3)	346.7 * (47.6)
Femur	288.8 (22.8)	113.7 (62.0)	352.0 * (118.8)	114.2 (9.9)	86.0 (23.1)	47.2 (10.6)	35.3 (11.0)	39.0 (7.0)	19.7 (2.9)	27.0 (5.0)	7.3 (0.6)	12.2 * (1.0)
Mesenteric fat	-	73.0 23.5)	84.3 (28.4)	-	-	-	(25.7 (12.4)	30.7 (9.4)	-	-	3.7 (0.6)	6.3 (2.3)
Testes	-	8.5 (2.1)	-	-	-	-	12.8 (16.3)	-	-	-	5.7 (1.1)	-
Ovary	-	-	936.0 (31.0)	-	-	-		323.0 (73.4)	-	-	-	98.7 (29.7)
Muscles	-	53[illegible].3 (375.8)	368.3 (190.0)	-	-	-	45.7 (27.2)	90.0 (45.1)			11.0 (7.0)	13.3 (7.5)
Eyes	-	155.3 (70.3)	244.7 (74.4)	-	-	-	42.7 (13.6)	41.8 (32.0)	-	-	15.7 (5.5)	18.3 (3.5)
Brain	-	17.7 (1.1)	18.7 (2.3)	-	-	-	6.1 (3.5)	6.0 (1.0)	-	-	2.8 (0.2)	3.3 (0.8)
Brown Fat	-	-	-	-	-	-	273.7 (53.5	214.0 (74.7)	-	-	112.0 (24.2)	96.7 (20.8)

* Statistically significant difference at p = 0.05

L. Report #25

1. Title:
Radioimmunoassay of Mitoxantrone

Investigators:
G. Nicolau
Lederle L.L.C.
Pearl River, NY

2. Objective:
To develop a sensitive and specific assay for mitoxantrone, since the sensitivity of current available methods is not low enough to provide meaningful pharmacokinetic data.

4. RIA in Rat Serum:
Antiserum from rabbits immunized with mitoxantrone-BSA were prepared. Tritiated mitoxantrone (--- Ci/mmole) was used as a radio-tracer ligand. Standard curves were linear in the concentration range of 75-2500 pg/ml at an antiserum dilution of 1:15,000. Assay sensitivity is 75 pg/ml in 0.5 ml serum samples. The cross-reactivity of metabolite A was $\leq$ 1% at concentrations up to 10 ng/ml serum.

14 rats were dosed at 0.5 mg/kg (iv). Blood was collected at 0.25, 0.5, 1, 2, 4, 8, and 24 hours after dosing. Concentrations of mitoxantrone in rat serum are summarized in Table 25.1. The lower values obtained by RIA for the unchanged drug as compared to total radioactivity data indicate the presence of metabolite(s) in the rat serum.

4. Conclusion:
The Division of Biopharmaceutics finds Report #25 acceptable.

TABLE 25.1

Concentrations of Mitoxantrone in Rat Serum Following Intravenous Administration of 0.5 mg/kg

Time After Dose (Hours)	Serum Concentrations	
	Total Radioactivity (ng equiv./mL)	Unchanged Drug (RIA) (ng/mL)
0.25	N.D.	45.70
0.5	29	33.59
1.0	20	9.01
2.0	11	3.88
4.0	12	2.49
8.0	7	0.70
24	6	0.45

Values are the means from two rats/time, except that at 0.25 hours (1 rat).

The lower values obtained for unchanged drug by RIA at later times, as compared to total radioactivity data indicate the presence of radiolabeled metabolite(s).

M. Report #47

1. Title:
"The Effect of Dose Level on the Serum and Tissue Concentration of ^{14}C-Mitoxantrone in the Dog".

2. Investigators:
V.K. Batra et. al
Lederle Lab
Pearl River, NY

3. Objective:
To determine dose proportionality of mitoxantrone in serum and tissues in dog.

4. Study Design and Procedure:
18 dogs were divided equally into 3 groups. One group was dosed at 0.05 mg/kg (iv), one group at 0.1 mg/kg and one group at 0.2 mg/kg of ^{14}C-Mitoxantrone. Blood in 3 dogs per group were collected from 0.5 to 60 days after dosing. The remaining dogs per group were for determining tissue drug concentrations at days 10 and 60 after dosing.

5. Study Results:
1. The radioactivity concentrations in serum, whole blood, and most of the tissues increased proportionally with dose, possibly suggesting linear pharmacokinetics of the drug over a dosing range of 0.05 to 0.2 mg/kg.

2. About 80% of the total radioactivity was found in red blood cells.

3. The biological elimination half-life of total radioactivity calculated from the blood concentration data following the 0.1 and 0.2 mg/kg dose was 36 and 39 days respectively (Table 47.1 & 47.2).

4. The elimination half-life of total radioactivity in various tissues is shown in Table 47.2. The mean t 1/2 = 47 days.

6. Conclusion:
The Division of Biopharmaceutics finds Report #47 acceptable.

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Table 47.1

Serum and Whole Blood Concentrations in the Dog Following Intravenous Administration of 0.3 mg/kg of ^{14}C-Mitoxantrone

Time (hrs.)	Dog 85647		Dog 46706		Dog 46714	
	Serum	Whole Blood	Serum	Whole Blood	Serum	Whole Blood
0.5	19.0	149.6	16.4	107.5	24.4	170.0
1	10.9	53.1	8.2	42.7	13.2	63.7
2	5.6	3..6	5.2	26.8	7.7	35.1
3	4.5	20.8	3.9	18.5	5.7	26.2
4	3.8	16.5	3.4	15.9	4.5	20.2
6	2.9	12.6	2.9	12.9	3.8	14.9
8	2.7	11.0	2.1	10.7	4.7	12.3
12	2.1	3..	1.7	8.9	2.6	10.2
24	1.4	..	1.3	3.6	1.8	5.5
36	1.3	4.3	1.5	2.2	2.0	5.1
48	1.6	3.8	1.4	2.9	1.7	4.0
72	.8	2.7	.97	2.2	1.4	3.4
96	.9	1..	.9	2.6	1.2	2.6
120	.7	1.6	.8	2.0	.9	2.4
156	.6	2.0	.5	1.5	-	2.5
192	.7	1.3	.6	1.4	.6	1.5
216	.7	1.5	1.2	1.3	.5	.8
240	.7	1.1	-	1.1	-	1.0
360	.63	.9	.50	1.0	1.67	1.1
528	.66	.6	.47	.75	.53	.9
720	.53	1.4	.38	.5	.46	.8
840	.61	.7	.36	.7	.28	1.3
936	.32	.6	.28	.4	.44	.5
1080	.28	.5	.25	.4	.33	.5
1200	.36	1.2	.26	.7	.28	.97
1272	.25	.5	.25	.3	.24	.4
1440	.25	.6	.2	.3	.31	.4

- = below assay sensitivity (0.3 ng/mL)

t½

39 days

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Table 47.2

The Elimination Half-Life of Total Radioactivity in Various Tissues Following Intravenous Administration of ^{14}C-Mitoxantrone

Tissue	Dose, mg/kg 0.05	0.1	0.2	Mean Half-Life (hrs)	Tissue	Dose, mg/kg 0.05	0.1	0.2	Mean Half-Life (hrs)
Adrenal	28.807	19.052	28.0[illegible]	25.307	Pancreas	--	72.156	101.166	87.01[illegible]
Bile	49.867	14.131	18.614	27.537	Pituitary Gland	--	20.872	17.[illegible]	19.16
Bladder	--	16.698	22.813	19.76	Prostate	29.854	16.818	25.0[illegible]	23.901
Bone Marrow	47.042	51.224	35.780	43.362	Retina	45.651	83.023	--	64.50
Brain	46.796	38.543	219.867	101.735	Salivary Gland	17.998	17.748	15.204	16.983
Breast	--	14.741	21.759	86.5	Sclera	9.899	24.[illegible]	647.76	47.850
Fat	72.666	42.328	17.135	42.377	Spinal Cord	--	89.157	--	[illegible]
Gall Bladder	--	66.019	78.328	72.17	Skin	94.758	18.161	14.85[illegible]	45.851
Heart	13.624	21.653	26.915	20.741	Small Intes[illegible]	16.083	13.755	108.520	[illegible]
Iris	--	--	--	--	Spleen	21.143	24.186	16.619	22.183
Kidney	20.629	22.651	42.276	28.452	Stomach	31.[illegible]	70.[illegible]	48.751	36.934
Large Intestine	16.468	14.640	14.250	15.053	Testes	23.[illegible]		1[illegible]	[illegible]
Liver	519	22.469	64.588	57.192	Thymus	16.[illegible]	[illegible]	14.69[illegible]	16.676
Lymph nodes	--	50.356	27.[illegible]	38.9	Thyroid	[illegible]	--	-	[illegible]
Lung	--	14.663	17.434	16.05	Tongue	17.[illegible]	15.719	20.[illegible]	17.752
Muscle	13.397	13.599	16.982	14.659	Tonsil	2[illegible] 658	19.991	19.625	21.658
Ovary	311.597	37.679	266.880	205.052	Uterus	-	86.237	29.617	[illegible]
					Whole Blood	-	36.129	--	36.129

-- Could not be determined (these values were not included in calculating the mean half-life

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Lederle Laboratories
Pearl River, N.Y. 10965

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Scientific Services Section

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Table 50.1

Concentration of Radioactivity in Selected Tissues of Adult Dogs 24 Hours After 5 Consecutive Daily Intravenous Doses at 0.1 mg/kg ^{14}C-CL 232,315 and 10 Days After A Single Intravenous Dose at 0.2 mg/kg ^{14}C-CL 232,315

Tissue	ng/g ^{14}C-CL 232,315 Equivalents							
	B8368[a]				B8367[b]			
	3902(m)		3907(f)		46710(m)		46711(f)	
	Homog.[c]	Whole Tissue[d]	Homog.	Whole Tissue	Homog.	Whole Tissue	Homog.	Whole Tissue
Heart	1270	941	922	761	238	191	605	513
Lung	1350	1030	460	438	333	247	668	414
Spleen	2050	2300	3480	3920	1790	1480	1700	1050
Kidney	4220	3860	4160	3350	1060	933	2490	1960
Liver	2950	2650	1980	1830	1440	1440	1590	849
Pancreas	2690	1800	1890	1540	788	388	1030	958
Stomach	618	453	642	676	85	87	113	113
Sm. Intestine	404	213	296	312	68	64	111	95
Lg. Intestine	315	141	227	190	100	45	69	89
Testes	79	100	-[e]	-	66	24	-	-
Ovaries	-	-	S.L.[f]	158	-	-	177	75
Uterus	-	-	201	151	-	-	155	149
Mammary Gland	-	-	154	83	-	-	67	40

a - Five daily consecutive intravenous doses at 0.1 mg/kg ^{14}C-CL 232,315. Tissues 24 hours after last dose.

b - A single intravenous dose at 0.2 mg/kg ^{14}C-CL 232,315. Tissues 10 days after dose.

c - Portion of sample homogenized before assay

d - Assay performed on a weighed portion of tissue (F.R.29:35-72, 1-27)

e - Not available

f - Sample lost

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Scientific Services Section

P.R. VOLUME 29

Table 50.2

HPLC Elution Profile Assay of the Radioactivity as ^{14}C-CL 232,315 in Selected Dog Tissues 24 Hours After 5 Consecutive Daily Doses at 0.1 mg/kg ^{14}C-CL 232,315 and 10 Days After a Single Intravenous Dose at 0.2 mg/kg ^{14}C-CL 232,315

	Percent ^{14}C-CL 232,315 in HPLC Eluate				
Tissue	2902(m)[a]	3907(f)[a]	46710(m)[b]	46711(f)[b]	Av. ± S.D.
Heart	89	92	69	82	83 ± 10.2
Lung	94	86	84	95	90 ± 5.6
Spleen	78	88	59	92	79 ± 14.7
Kidney	85	91	84	93	88 ± 4.4
Liver	89	88	54	89	81 ± 17.3
Pancreas	91	82	93	89	89 ± 4.8
Stomach	83	80	INC[c]	77	80 ± 3.0
Sm. Intestine	81	79	INC	82	81 ± 1.5
Lg. Intestine	85	53	70	82	73 ± 14.5
Testes	46	-[d]	80	-	63 ± 24.0
Ovaries	-	S.L.[e]	-	S.L.	
Uterus	-	83	-	41	62 ± 29.7
Mammary Gland	-	88	-	INC	88 ± 0

a - Five daily consecutive doses at 0.1 mg/kg ^{14}C-CL 232,315. Tissues 24 hours after last dose.

b - A single intravenous dose at 0.2 mg/kg ^{14}C-CL 232,315. Tissues 10 days after dosing.

c - Results inconclusive

d - Not available

e - Sample lost

9/1/c

635

N. Report #50:

1. Title:
"Composition of Tissue in the Dog After Single and Multiple IV Administration of ^{14}C-Mitoxantrone".

2. Investigators:
V.K. Batra et. al
Lederle Lab.
Pearl River, NY

3. Objective:
To assess the composition of ^{14}C-Mitoxantrone related material in tissues of dog.

4. Study Design and Analytical Procedures:
For dogs (N=2) receiving 0.1 mg/kg of ^{14}C-Mitoxantrone for 5 consecutive days, the tissues were collected 24 hrs after the last dose. For dogs (N=2) receiving a single dose of 0.2 mg/kg the tissues were collected at the end of 10 days. Radioactivity were measured by scintillation counting. Drug related material were isolated by HPLC and TLC.

5. Study Results:
Table 50.1 summarizes the concentration of radioactivity found in 13 tissues selected for study. Table 50.2 summarizes the percentage of unchanged drug in these tissues. With the exception of testes and ovaries which showed low measurable radioactivity (therefore making calculations unreliable for these two particular tissues), 73 to 90% of the eluted radioactivity is attributed to the parent drug residing in the 11 tissues examined.

6. Conclusion:
The Division of Biopharmaceutics finds Report #50 acceptable.

III. Overall Deficiencies:

1. Inadequate estimation of pharmacokinetic parameters and lack of assay validation data.

Due to assay sensitivity limitations of the HPLC procedures adopted and difference in the number and times of sample collection, Reports #40, 41, 39 and 42 describe triphasic kinetics for mitoxantrone whereas the 3 submitted literature articles (Reports #36, 37 and 38) describe biphasic kinetics for it. The terminal half-life estimated in these studies varies from 1 to 38.6 hrs which is inconsistent with the urine excretion data as well as tissue drug levels found in autopsy specimens. The mean half-life estimated by RIA is 12.4 + 3.1 days (N=6). However, only 1 patient (the Henderson patient) had sufficient data points (6 points) to obtain a statistically significant correlation of LnCp versus time. In addition to the overall apparent underestimated terminal half-life, lack of assay validation data were also found in some of these studies. It is suggested that the sponsor contact the Division of Biopharmaceutics for details with regard to the deficiencies for each individual study report.

2. Inconclusive evidence with regard to the effect of mitoxantrone accumulaton on the pharmacokinetics and metabolism of the drug.

From the available data reported in this submission (terminal t 1/2 = 12.4 days by RIA, autopsy results and limited excretion of the drug from the body) it is evident that mitoxantrone has a high probability for accumulation in the body using a dosing schedule of 12 mg/m^2 every 3 weeks. (The proposed dosage regimen in the labeling is 14 mg/m^2 every 3 weeks). Although the investigator of Report #40 claimed that repeated dosing for as many as 12 courses (patient #007) had no noticeable effect on the calculated pharmacokinetic parameters, the Division of Biopharmaceutics finds that this argument is less convincing due to the following reasons:

a) Lack of a well controlled study for patient #007 (multiple dose data).

b) Limited number of (5) patients were used.

c) Variability of individual pharmacokinetic data.

Additionally, in analyzing the pattern of plasma disappearances of ^{14}C and mitoxantrone in Report #40, it is noted that patients who had not received any prior mitoxantrone treatment fell into one group (I) and the 3 patients (#005, 006, 007) who had prior mitoxantrone treatment fell into another group (II), The group I patients had slower declining rate of ^{14}C and had a final ^{14}C/mitoxantrone concentration of 5:1. On the other hand, the group II patients had ^{14}C declining rate that paralleled the mitoxantrone declining rate and there was little difference between the two determined concentrations. The urine excretion patterns (^{14}C vs. mitoxantrone) for each patient agreed with his/her plasma patterns. Of particular interest is the finding that for patient #007 who had 12 prior mitoxantrone treatments, the total amount of mitoxantrone recovered in each urine collection period was equal to the amount of total ^{14}C recovered suggesting that no metabolites were present. Whether these observations imply alteration of mitoxantrone metabolism during the course of repeated dosing or simply reflect common characteristics found in the 3 patients in Group II cannot be concluded based on the data reported in this submission. You should provide information to address this issue.

3. Conflicting information with regard to the effect of hepatic disfunction on the pharmacokinetics of the drug.

Your Report #41 and #39 suggest that the pharmacokinetics of mitoxantrone in patients with abnormal liver function are not significantly different from patients with normal liver function. However, Report #36 found that patients with abnormal liver function had a CLT less than one half that found for patients with normal liver function (100.7 vs 238.7 ml/kg/hr) The terminal t1/2 of the drug was almost double in those patients with liver disfunction (70.7 vs. 37.4 hr). However, due to (a) the insensitivity of assay procedure used (pharmacokinetic parameters were estimated from plasma data only covering in 0-10 hr post-drug administration). and (b) the lack of individual plasma data for an accurate evaluation of Report #36, no conclusion on this issue can be made at this time.

4. The formulation to be marketed as identified in the package insert contains acetate buffer (pH~3.7). Pharmacokinetic studies for this formulation are not available and the pharmacokinetic studies provided in this submission were conducted using a sodium metabisulfite (0.2% W/V, MRWO 724,480) formulation. Please provide pH-solubility profile (range pH 3-8) of the bulk drug.

IV. Conclusion:

The Division of Biopharmaceutics finds the pharmacokinetic studies conducted with the 0.2% w/v sodium metabisulfite containing formulation (MRWO 724,480) submitted on May 15, 1984 not acceptable. The above Overall Deficiencies (1-4) and Conclusion should be forward to the sponsor for comment by them.

Ko-yu Lo 3/4/86

Ko-Yu Lo, Ph.D.
Pharmacokinetics Evaluation Branch

RD Initialed by John P. Hunt
FT Initialed by C.T. Viswanathan, Ph.D. CTV 3/4/86

cc: NDA 19-297 Orig., HFN-150, HFN-225(Lo), HFN-344(Turner), Drug, Chron, and FOI files.

KYL:smj:4251x:02-25-85

CHEM

REV

CHEMIST'S REVIEW #4

Date Completed: 12/11/87

A. 1. NDA 19-297

Applicant: Ledeule Laboratories
Pearl River, New York 10965

2. Product Name:

Proprietary: Novantrone Injection
USAN: Mitoxantrone Hydrochloride

3. Dosage Form and Route of Administration:

Sterile, aqueous solution for IV infusion after dilution, for single dose use. 10 mL, 12.5 mL and 15 mL vials, 2 mg/mL.

4. Pharmacological Category:

Antineoplastic agent.

B. 1. Initial Submission: 5/18/84
2. Amendment: 12/10/87

B. Remarks:

The applicant submitted the draft package insert for NDA 19-297 Novantrone for Injection. The evaluation of the submitted document is given below, in the Conclusions and Recommendations section.

C. Conclusions and Recommendations:

~~The title of the PI should read "Novantrone~~ Injection".

The Agency requested ~~in~~ in the letter of 5/30/86 that the storage condition and acceptable length of storage time for the diluted drug be included in the "Dosage and Administration" section of the package insert.

The proposed labeling submitted on 12/10/87 contains the following paragraphs in the "Dosage and Administration" section:

NOVANTRONE solution should be diluted to at least 50 ml with either Sodium Chloride for Injection (USP) or 5% Dextrose for Injection (USP). This solution should be introduced slowly into the tubing as a freely running intravenous infusion of Sodium Chloride for Injection (USP) or 5% Dextrose for Injection (USP) over a period of not less than 3 minutes. Unused infusion solutions should be discarded in an appropriate fashion.

NOVANTRONE may be further diluted into Dextrose 5% in Water, Normal Saline or Dextrose 5% with Normal Saline and remains stable for up to 7 days from time of admixture; NOVANTRONE solution stability is maintained either alone or when further diluted, for up to 7 days when refrigerated. DO NOT FREEZE.

The first paragraph concerning the dilution of the drug is acceptable, however the underlined portion of the second paragraph claiming stability for seven days for the diluted solutions should be removed from the package insert. After consultation with the Microbiologist Dr. Vivien Greenberg who expressed concern about the microbiological quality of diluted solutions of this drug stored for seven days, especially solutions containing dextrose the following recommendations are made:

(1) The title of the PI should read Novantrone Injection wit Novantrone for Injection.
(2) The underlined portion of the second paragraph should be deleted.
(3) It is recommended that the applicant state in the PI that diluted solutions of the drug be used immediately.
(4) If the applicant proposes at a later date, to include a section in the PI claiming an appropriate storage time for the diluted solutions, supportive chemical and microbiological data need to be provided.

Eva Thorfinson

MED

REV

NDA 19297 MOR #2
DRUG MITOXANTRONE (LEUKEMIA,BREAST) 12/9/87

LEUKEMIA

The initial review of the two prospective randomized controlled trials in ANLL which were submitted 10/28/87 generated several requests for additional information which has been supplied by Lederle.

An additional confirmatory intention to treat analysis was requested for study 3-74, a comparison of mitoxantrone plus ara-c vs. daunorubicin plus ara-c in ANLL. The primary analysis used all patients randomized with the correct diagnosis who actually received therapy. There were six patients initially randomized to the mitoxantrone containing arm who were never treated and hence excluded from the demoninator of the efficacy analysis. There were two such patients on the daunorubicin containing arm. Three of those randomized to mitoxantrone who were not treated were not strictly eligible. Both patients who were not treated with daunorubicin were eligible.

The following table compares the initial analysis with the intention to treat analysis that excludes only those patients with an incorrect diagnosis.

	Mitoxantrone + Ara-c		Daunorubicni + Ara-c	
	Initial	New	Initial	New
Total	98 (100)	104 (100)	102 (100)	104 (100)
Censored Alive	36 (37)	36 (35)	34 (33)	35 (34)
Lost to follow	1 (2)	2 (2)	2 (2)	2 (2)
Dead	61 (62)	66 (63)	66 (65)	67 (64)
CR	62 (63)	62 (60)	54 (53)	54 (52)

Hazard Ratio C:M [95% CL]	Initial	1.14 [0.80,1.62]
	New	1.10 [0.78,1.54]

The addition of the patients who were not treated does not alter the overall results of the study. The response rate and overall survival are quite comparable in study 3-74.

The question of possible differences in toxicities as measured by changes in laboratory parameters that were not put to formal statistical testing can be addressed in labelling where the more

Lederle has satisfactorily addressed the statistical issue raised in my initial review of this study. This study provides adequate demonstration of the safety and efficacy of mitoxantrone when used in combination for ANLL in adults.

A similar request for an additional intention to treat analysis was made for the second randomized comparison of mitoxantrone plus ara-c vs. daunorubicin plus ara-c in ANLL (3-603, the international study). The primary analysis excluded three patients initially randomized to the mitoxantrone containing arm because they did not receive treatment. There were no comparable patients in the daunorubicin containing arm.

An additional analysis was requested in which the patients who were treated at the two centers in the Pacific region where the randomization process was not properly carried out are excluded. These centers were at Hong Kong and Taiwan where a total of 31 of the 237 patients randomized with the correct diagnosis were not treated. This new analysis was requested only to provide confirmation that major qualitative differences in outcome would not appear. Removal of these patients from the overall analysis does somewhat reduce the power of the test to demonstrate differences and if the new analysis continues to demonstrate comparable efficacy I would not take this result as providing more assurance of comparability. Thus, only negative results are really being sought. If bias was introduced that favored mitoxantrone in a significant way a less favorable result might become apparent in the new analysis that excludes these patients.

The new analysis that includes all patients randomized with the correct diagnosis was not possible regarding overall survival since the three patients excluded from the initial analysis are all censored at or shortly after baseline. An assumption must be made that this exclusion will not materially add bias in favor of mitoxantrone. Since the number of subjects involved is small this is reasonable. The inclusion of these three patients in the denominator for the response rate slightly reduces the response for the mitoxantrone containing arm from 50% [41%,59%] to 49% [40%,58%]. The response on the daunorubicin containing arm remains at 51% [42%,60%]. These response rates remain virtually indistinguishable (p= .74).

The analysis performed when the patients who were treated at Hong Kong and Taiwan are excluded resulted in a response rate of 49% [39%,59%] on mitoxantrone and 52% [42%,61%] on daunorubicin. The hazard ratio C:M in the initial analysis was 0.84 [0.6,1.16] and with the patients excluded from Hong Kong and Taiwan the ratio is 0.84 [.60,1.22]. Although these analyses reduce the power of the test no additional evidence is forthcoming that the response rates or overall survival are indeed different. This new analysis is not further supportive, rather it simply does not provide evidence that substantial bias was introduced favoring

Because the overall survival in study 3-603 slightly favored the daunorubicin containing arm and since the lower bound of the 95% confidence limit for the hazard ratio is 0.60 further analyses were performed by Lederle in an attempt explain this difference and the variance from study 3-74. The difference in survival between the arms in study 3-603, in spite of comparable complete response rates, might be explained by several factors. There was an excess of eight deaths on the mitoxantrone containing arm in the consolidation phase of therapy. Reasons for this finding were sought. Of these eight deaths five were attributed to infection, two to hemorrhage, and one to acute renal failure and infection. In the U.S. study there were two consolidation deaths on mitoxantrone and one on daunorubicin, all due to infection. Deaths during periods of hypoplasia may well be related to the adequacy of antibiotic and hematologic support. An hypothesis was generated that the adequacy of antibiotic support might explain these results.

Firstly the degree of myelosuppression was compared in consolidation in study 3-603 between the arms. There appeared to be a regional difference in the degree of myelosupression. Median absolute granulocyte nadirs for Europe, Latin America and Pacific were 0.1, 0.7, and 1.0 on daunorubicin whereas all regions uniformly had nadirs of 0.1 or less on mitoxantrone. Also more patients on mitoxantrone appeared to be at risk for hemorrhage on mitoxantrone. These differences in the degree of myelosuppression noted between the arms in consolidation in the Latin America and Pacific regions were not seen in the U.S. study where both regimens appeared equally myelosuppressive and in fact similar to the European areas. There is no explanation for the apparent lessened myelosuppressive effect of daunorubicin seen in consolidation in Latin America and the Pacific.

If antibiotic support was not adequate the difference in myelosuppression seen in the Latin American and Pacific regions might be reflected in outcome. An analysis was made of the adequacy of antibiotic support in both study 3-74 and 3-603. Adequate support was defined as per the recommendations in the AMA Drug Evaluation Handbook as to the proper selection of drug and according to the Medical Letter as to the appropriate dosing.

During consolidation in study 3-603 32/45 patients on mitoxantrone had both neutropenia and signs or symptoms of infection while only 7/34 on daunorubicin had such findings. 81% of those on mitoxantrone and 71% on daunorubicin had suboptimal antibiotic support. Of the six patients who died of infection in consolidation on mitoxantrone 5 had inappropriate or no antibiotics given.

The use of antibiotics was also examined in the U.S. study 3-74. 32 of the 62 who entered consolidation on mitoxantrone and 23 of the 54 on daunorubicin developed absolute granulocyte counts less than 500 and signs or symptoms of infection. Two on

mitoxantrone and one on daunorubicin died of infection. During consolidation suboptimal antibiotic coverage was given to 53% on mitoxantrone and 47% on daunorubicin. Thrombocytopenia and neutropenia appeared similar in both arms of the study.

These data support the hypothesis that the poorer outcome during consolidation in study 3-603 might be related to an unexpected decrease in the amount of myelosuppression on the daunorubicin containing arm in a setting where antibiotic care may not have been optimal. The U.S. study supports this hypothesis and suggests that the degree of myelosuppression seen during consolidation can be better supported if antibiotic coverage is more adequate. This would explain why the overall survival in study 3-603 favors daunorubicin in spite of the fact that the complete response rate during induction is similar.

These data do not prove this hypothesis however as they are derived from a retrospective analysis of the data. It is interesting to note that there was also a slight imbalance in study 3-603 in the number of deaths during induction during a phase of marrow aplasia or hypoplasia (9 on mitoxantrone and 4 on daunorubicin) which would further suggest that the difference in overall survival might be in at least partly have been reduced had adequate antibiotic support been available.

Study 3-603 provides adequate evidence that mitoxantrone in combination with ara-c is effective in inducing complete response in ANLL. The overall survival was similar as well, but the lower 95% confidence limit of the hazard ratio was only .6 which allows a conclusion that the survival time on mitoxantrone could only 60% of that on daunorubicin. The confidence in the overall survival data generated in study 3-603 is reduced by the confounding factors of an unanticipated difference in the myelosupressive response of patients in Latin America and the Pacific regions to daunorubicin and to what is considered to be inadequate antibiotic coverage. The survival data from study 3-603 does not confirm nor does it deny the conclusion reached regarding comparable survival in study 3-74. Furthermore, the patient population studied in 3-74 more closely represents the U.S. population with regard to possible confounding factors such as concurrent infections and possible racial differences in drug metabolism.

The regulatory requirement for approval is met by the demonstration of comparable response rate with daunorubicin plus ara-c in both 3-74 and 3-603 and by the demonstration of comparable survival in study 3-74. Therefore this NDA for the use of mitoxantrone in combination with ara-c for use in the treatment of adult ANLL should be approved.

The indications section of the labelling should focus on the initial therapy of ANLL since the utility of consolidation therapy in ANLL is not established. However, since consolidation was [illegible] treated in these controlled clinical studies its use

should be described for those patients for whom its use will be made. However consolidation therapy should not be specifically be recommended. The adverse reactions section of the labelling should emphasize the experience described in the U.S. study because these events appear to be better documented in that study. Additional adverse events seen in the international study can be added.

[redacted]

[redacted]

RECOMMENDATIONS

1.)This NDA for the use of mitoxantrone in combination with ara-c for use in newly diagnosed adult patients with ANLL should be approved. The request for approval for use of mitoxantrone as a single agent or in combination with ara-c for relapsed or refractory ANLL should not be approved.

2.)Labelling must be resubmitted for review prior to approval.

[redacted]

4.)The division of scientific investigations should audit the U.S. Study 3-74, as it is most pivotal.

Gregory P Burke

Gregory Burke M.D.,Ph.D.
12/9/87

John L Johnson MD 12/10/87

cc
NDA 19297
HFN 150/BURKE
HFN 150/KE
HFN 150/SINDELAR
HFN 150/DIV FILE
HFN 340

PHARM REV

A

Subsequent submission review of NDA 19297
Review #3
Date of submission:3/19/87, received by HFN-150 on 3/23/87
Date of review completed: 8/4/87

APPLICANT: Lerlerle laboaratories
Pearl River, New York 10985

DRUG: Novantrone (mitoxantrone hydorchloride)

DRUG CATEGORY: Antineoplastic agent.

MATERIAL REVIEWED:

The applicant resubmitted three complete carcinogenicity data containing summary tables and formats specified by the Division of Biometrics. The previous submission did not provide a detail report for the two mouse studies. Additional pharmacology and toxicology studies(acute toxicity and dermal tolerance) were included.

Carcinogenicity study:
The route and frequency of administration was intravenous once every 21 days for 2 years.

Rats: Similar conclusion was reached as that of the original submission. The occurrence of neoplastice lesions was generally low with the exception of an increased incidence of external auditory canal tumors noted in intermediate-dose males and high dose males and females (statistically significant). Fibroma was also observed stastistically significantly in high dose females.

Mice: A co lte neeplastic finding was submitted. The prevalence and trend P-values of common tumors of the two studies is listed in the following five pages (table 1-5). The prevalence of less common tumors was less than 2; thus, they are listed in the review. The data indicated that occurrence of lesions was low except that a significant increase in incidence of hepatocellular carcinoma and adenoma was observed in the first mouse study (0.1, 0.2, and 0.4 mg/kg).

Pharmacology study:Performed by Lederle (Japan) Ltd.
Respiratory and cardiovascular systems: The compound did not have any remarkable effect on respiratory-cardiovascular system in the proposed human dose of 12-14 mg/m^2 (0.4 mg/kg). At a dose of 10 mg/kg, it caused a decrease in the femoral arterial blood pressure, femoral aterial blood flow, heart rate and respiratory amplitude and an increase in repiraltory rate in anesthetized dogs. At the same dose level, it decreased the QRS amplitude and flattered T wave on EKG.

Autonomic nervous system: At 10 mg/kg, it suppressed the contractions of the nictitating membrance induced by electrical stimulation of both pre- and post-ganglionic nerve of superior cervical ganglion in anesthetized cats. At lower doses (0.4 and 4 mg/kg) , it was inactive in the testing systems. The responses to acetylcholine, histamine and barium chloride in isolated quinea pig ileum, the responses to oxytocin in isolated rat uterus and the respone to isoproterenol in isolated qunea pig tracheal chain were inhibited.

CNS:The CNS effects were studied in mice, rats, cats and rabbits. In the models used in the study, there were not noteworthy effects on the CNS at dose up to 10 mg/kg. However, mice showed a decrease of spontaneous movement and piloerection with iv administration of 40 mg/kg.

GI and other systems: At doses of 0.4-10 mg/kg given intravenously, it had no influence on the GI transport in mice or the biliary secretion in rats. At doses of 4 and 10 mg/kg, it decreased the volume of gastric juice and the ionic output. It had no effect on the twitches of the striated muscle of diaphragm induced by electrical stimulation of the phrenic nerve. It caused prolongation of prothrombin time at doses of 0.4, 4 and 10 mg/kg.

Toxicology: Performed by Ledrele(Japan) Ltd.
Acute oral toxicity study in mice:Carried out by Lederle (Japan).
Dose levels: 40 to 1280 mg/kg. po.
Observation period: 21 days.
Toxic signs: Decrease in locomotor movement, rough hair, hunch back postion and smudge of perianal area, and death.
The deaths were occurred on days 5-12.
Decrease in body weight was observed at doses of 640 mg/kg.
Pathology: Lesions were found in the GI, thymus, spleen and bone marrow in the mice receiving 320 mg/kg or more.
LD50 was 501.6 and 552.4 mg/kg for males and female, respectively.

Acute subcutaneous toxicity study in mice:
Dose levels:7.3 to 35 mg/kg. sc.
Observation period: 21 dyas.
Toxic signs: Salivation, rough fur, paleness and perinal smudge. alopecia and death.
Bod weight loss was observed and appeared to show a trend toward recovery.
LD 50 was 19.7 and 22.3 mg/kg for males and females, respectively.
Pathology:Lesions at the injection site were noted. Lymphoid atrophy, pale bone marrow, and mucosal thickening of nonglandular stomach were seen.

Acute sc toxicity study in rats:
Dose levels: 2.0 to 10.7 mg/kg
Observation period: 21 days.
Toxic signs: Nodules at the infecion site, rough fur, decrease in locomotor movement, paleness, and death were seen. Death occurred in animals geven 5.5 mg/kg and above.
Gross findings: Lymphoid atrophy, discoloration of visceral oragan and subcutaneous region.
LD50 could not calculated for males (estimated to be 5.5 to 7.7) and was 6.7 mg/kg for females.

Acute toxicity study by the IV administration in rats in comparion with the metabolites [redacted]
The result indicates that the acute toxicity of the two metabolites was found to be less than that of mitoxantrone. An iv dose of mitoxantrone of 5 mg/kg resulted in 30-50 % mortality. No deaths were observed following equimolar doses of the two metabolites. The histological examination revealed similar lesions previouly recorded of mitoxantrone. No drug-related findings were recorded at necropsy for animals treated with the two metabolites.

Dermal tolerance study by single or multiple applicatin to rats:
Performed by Cyanamid Italia S.p.A (Italy).
Dose level: 8 mg/kg/d for one day or 2-4 days.
No effect was observed in the study.

Mutagenicity study: A published paper (Drug Research 36,1375-1379, 1986).
Mitoxantrone produced significant clastogenic effet in bone marrow of rats treated for 5 days at 0.5 mg/kg ip. It produced increases in DNA repir in the rat hepatocyte UDS test and increased SCEs in CHO cells and mutant frequencies in mouse lymphoma assay. In the cell transformation test, mitoxantrone did not produce significant increases in type II or type III tranformed foci. In the dominant lethal test in rat(2 mg/kg/d ip), it affected matings of treated males; however, total implantation as well as early death were unaffected.

EVALUATION AND COMMENTS:

A final evaluatin of carcinogenicity of mitoxantrone will be assessed upon the completion of biostatistical analysis of the studies. Since some positive mutagenic results were reported, a brief statement describing the results should be included in the Package Insert. It appeared that the doses used in the carcinogenicity studies were too high (high mortality at the end of the studies). High mortality of rats may suggest that the strain of rat may not suitable for long-term study.

Ching-Long Joseph Sun Ph.D.

R/D HFS Hirschler
J Rahman
12-16-87

2045E
cc:
Orig. NDA 19-297
HFN-150/Div. File
HFN-150/CJSun
HFN-150/AMSindelar
HFN-340
F/T:KE

DIV.

AUG 13 1985

Pharmacological and Toxicological Review

Original Summary

NDA 19-297

Date of Review Completed: 11/23/84
Date of Submission: 5/17/84
Date of Receiving: 5/24/84
Date of Assignment: 9/28/84

Sponsor: Lederle Laboratories
Pearl River, New York 10965

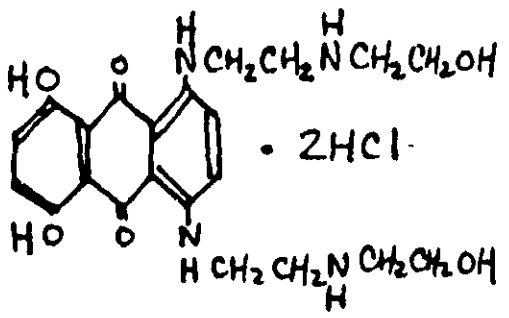
Drug: Novantrone^{R} (Mitoxantrone hydrochloride for injection).

Chemical Name: 1,4-Dihydroxy-5, 8-bis[[2-[(2- hydroxyethyl)]amino]ethyl] amino]-9, 10-anthracenedine dihydrochloride.

Molecular Formula: $C_{22}H_{28}N_4O_6 \cdot 2HCl$

M.W.: 517.4

Chemical Structure:



Drug Category: Antineoplastic agent to be used in the management of breast cancer.

Related Drugs: IND
IND

Proposed Marketing Indication:

For the treatment of locally advanced or metastatic breast cancer.

Preclinical Studies:

This submission of 166 volumes containing the following preclinical studies (Vol. 1.29-1.39).

Antitumor Activity:

Mouse leukemia system.
Mouse solid-tumor system.
Mechanism of Action.

Pharmacokinetics: Both in animal and human.

Excretion.
Distribution.
Plasma level.
Metabolism.

Toxicology and Pathology:

Mouse, LD50.
Rat, LD50.
Rat, single dose I.V. toxicity and effect on rat myocardium.
Dog, single dose I.V. toxicity.
Monkey, single dose I.V. toxicity.
Rat, daily for one month toxicity.
Dog, X5, I.V. toxicity.
Dog, X14 I.V. toxicity.
Dog, X5, 9 day recovery, 3 cycles toxicity.
Monkey, X5, I.V.
Monkey, X14, I.V.
Monkey, once/21 days, 2 cycles I.V.
Rat, I.V., once/21 days for 12 months.
Dog, 30 weeks I.V. intermitten dosing.
Monkey, 44 weeks I.V. intermitten dosing.
Rabbit, 21 weeks I.V. intermitten dosing.
Dog, I.V. toxicity intermitten dosing after Doxorubicin.

Genetic toxicity:

Microbial mutogenicity.
Unscheduled DNA synthesis.
Sister chromatid exchange.
Mouse lymphoma test.
Cell transformation.
Rat Cytogenetics.
Dominant lethal test.

Reproductive Toxicology and Teratology.

Carcinogenicity:

Mouse.
Rat.

Miscellaneous Studies.

Topical toxicity, rat and rabbit.
Ocular irritation, rabbit.
Dermal sensitization, guniea pig.
Combination toxicity, dog.
Heparin activity in vitro.

Pharmacology.

Antitumor activity:

Novantrone increased lifespan and number of long-term survivors in P388 (I.P., S.Q.), L1210 (I.P., I.V.). It was active against I.P. implanted B16 melanoma (I.P.) and was not effective against SC-implanted B16 melanoma. It is also active against ip implanted colon tumor 26 (I.P.). It was ineffective against Lewis lung cancer (I.V., S.Q.). It was ineffective in inhibition local tumor growth or in prolonging survival of Ridgway osteogenic sarcoma-bearing animals. Mitoxantrone has been reported to be active against CD8F1 mammary carcinoma and murine colon carcinoma 38 but inactive against human mammary, colon and lung xenografts in athymic mice.

Comparison with other antineoplastic agents: Tumors were implanted in mice I.P. and drugs were given I.P. Mitoxantrone was more effective than cyclophosphamide, 5-FU, MTX, cytosine arabinoside, vincristine, doxorubicin in P388 L1210, B16 and colon 26 except that doxorubicin was more effective against B16 melanoma.

Synergistic activity was demonstrated in mice in the treatment of L1210 leukemia when mitoxantrone was combined with cyclophosphamide, vincristine or thiotepa.

Mechanism of action:

Mitoxantrone binds to both DNA and RNA. Mitoxantrone was more potent than doxorubicin compared on an equimolar basis in inhibiting the uptake of ^{3}H-uridine and ^{3}H-thymidine by mouse lymphoma L15178Y cells in vitro.

Mitoxantrone inhibited proliferation of human colon carcinoma cells in vitro and induced nuclear aberrations. Mitoxantrone produced a decrease in mouse bone marrow cellularity and a cytocidal effect in both proliferating and [illegible] proliferating cultures. Mitoxantrone produced the greatest cytotoxicity [illegible] phase, it was also effective against cells in S phase or [illegible] investigation. It indicated that mitoxantrone was not [illegible] specific.

Effect on the cardiovascular system:

The responses to tyramine, epinephrine and angitensin were lowered by mitoxantrone. No change in heart rate and blood pressure were observed in spontaneously hypertensive rats after mitoxantrone. A slight increase in blood pressure did occur in cat with no changes in heart rate or EKG.

Pharmacokinetics:

Disappearance of radioactivity from blood, plasma, serum.

Animal - Pattern of disappearance is biphasic in rats with t1/2alpha = 2.3 hours, t1/2B = 11.83 days. In dogs, disappearance is also biphasic with half time of 3 and 20 minutes (report #32). Amount of radioactivity were detectable through at least 10 days (rats), 58 days (dogs) or 35 days (monkeys). The large volume of distribution and the large plasma clearance in the rat indicate tissue uptake of radioactivity. In monkey, the half-life during the first 2 hours is about 21-38 minutes, and 8.5 $\pm$ 3.7 days from day 1 through day 35.

Human - Based on several studies, the pharmacokinetic of mitroxantrone has been described as either biphasic or triphasic. The variability of mitoxantrone reflects the sensitivity limitations of analytical procedure, the timing of sample collection, and inter-subject variability evident within and among studies. However, all investigators characterized as a very rapid initial distribution phase followed by a relatively slow elimination phase or phases.

Mean Elimination t 1/2 (hours).

Report No.	α	β	Γ
35	0.25	24	ND
36.37	0.228	37.4	ND
38	very rapid	0.99	ND
39	0.060	0.42	2.86
40	0.10	1.04	38.6
41	0.04	0.28	3.27
42	0.068	0.33	8.90

[illegible].

[illegible] mitoxantrone and its metabolite (based on [illegible]) very slowly by both renal and biliary

excretion with biliary route predominating. Excretion of radioactivity in urine and feces 10 days after I.V. administration of ^{14}C-mitoxantrone is shown as follows:

	Rats (% of dose)	Dogs	Monkeys
Urine	16.8	5.7	10.9
Feces	66.2	56.8	57.9

In rats given ^{14}C-mitoxantrone orally, only 1.2% of total radioactivity administered was excreted in the bile, indicating the poor oral absorption. There were no significant differences in either the rate or extent of excretion of radioactivity between male and female rats by I.V. administration. In the rats, the elimination half-life was 12.1 days.

Human - A mean of 10.1% of administered radioactivity was recovered in urine in 96-120 hours. Sixty-five percent was unchanged mitoxantrone. Other reports indicated 3%, 6%, and 8.4% excreted as unchanged mitoxantrone. For comparison, following table shows the rate and extent of urinary and fecal excretion of mitoxantrone in rat, dog, monkey, and human.

Time(days)	Rat	Dog	Monkey	Human
	Urine (cumulative percent of dose)			
5	13.6	3.7	8.9	9.6
	Feces			
5	56.0	50.5	47.3	18.3

Tissue Distribution.

Animal - Some similarities in distribution and clearance can be seen among rats, dogs, and monkeys. In all three species, value on day 1 and 2 were highest in the bile, gallbladder (except rats), liver, spleen and kidneys. Slight amount of radioactivity (less than 50 mg/g) were seen in spinal cord, CSF, brain, cornea, aqueous humor, vitreous humor, testes (rat) and fetus (rat). data indicate that Mitoxantrone is rapidly distributed and half-life. All of the radioactivity present in the of rats at 6 hours and 5 days after a single I.V. was unchanged. However, in the liver 61% and 44% at 6 hours and 4 days was mitoxantrone, bution of radioactivity was indepent of dose in

In a study to assess accumulation potential, the data showed accumulation in most tissues in dogs.

Higher tissue levels of radioactivity were attained in rats following slow infusion than were attained following a single IV bolus dose; divided IV bolus doses resulted in relatively low tissue levels. Differences were substantial for heart, lungs, spleen, kidneys, stomach, and adrenal glands.

Placenta of 18-day pregnant rats exhibited a tendency to accumulate the drug followed by slow clearing from the tissue. The half-life for the elimination from placenta was approximated to be 22 hours. About 0.4% of the total concentration of the drug in the placenta was found in fetus. Only 7 ng/ml of 14C mitoxantrone was found in the amiotic fluid one hour after dosing, as compared to 1342 ng/g of tissue were found in placenta. There was no detectable amount of the drug in the amiotic fluid 6 hours after dosing. It appeared to indicate that the transfer of the drug from the placenta to fetus or the amiotic fluid is minimal.

Results indicated that intra-arterial administration can produce higher local tissue concentration than are achieved following an equivalent IV dose. Uptake of 14C-mitoxantrone in tumor tissue in mice inoculated IP with B16 melanoma were low relative to other tissue of the mouse. Sponsor suggested that it is due to a large necrotic core in tumor tissue.

Human - limited information concerning tissue distribution of mitoxantrone in [illegible] autopsy from one cancer patient contained concentration of [illegible] comparable to those present in the dog and monkey, suggesting [illegible] mitoxantrone attains high tissue concentration. Biopsy [illegible] obtain from patients after a single dose of [illegible] concentration of mitoxantrone, indicating a [illegible] tissues of human.

[illegible] ained 70.1% unchanged [illegible] lection period, unchanged [illegible] of biliary radioactivity, [illegible] of mitoxantrone, a major [illegible].4% in the 0-15 minute bile [illegible] utes to 6 hours). A mixture of [illegible] 8.6% of the 2 to 6 hours bile

Human - The cumulative urinary excretion of radioactivity was 6%. The patient urine contains three drug-related components, mono- and dicarboxyle acid derivatives and glucuronide conjugates.

Toxicology.

Acute toxicity studies.

Mouse: 10F and 10M were used for each dose level. LD10 and LD50 value are shown in table.

Route	Dosage Range (mg/kg)	Sex	LD10	LD50
IV	1.9-17.1	M	7.8	11.3
		F	7.1	9.7
IP	4.5-21.5	M	7.1	16.5
		F	2.3	19.7

In the mouse IV study, all deaths occurred at doses of 7.0 mg/kg or above. In the the mouse IP study, deaths occurred at doses of 2.9 mg/kg or above.

In both studies, signs of toxicity were: salivation, paleness, rough fur, hair loss, decreased body-weight gain, and body-weight loss. Additional signs of toxicity (IP) were abdominal distention, external abdominal staining; diarrhea, pertonitis and ascites.

LD50 values for mitoxantrone in each of the two vehicles (physiological saline and Acetate buffered formulation) were comparable.

Rat.

Acute lethality in rats.

Dosage Range (mg/kg)	Sex	No. of Rats/Dose	LD10 mg/kg	LD50 mg/kg
		10	3.5	4.8
		10	3.6	5.2
		10	6.2	8.0
		10	9.9	11.7
	M	10	422	682
	F	10	474	721

In IV and IP-dosed rats, signs of toxicity were: epistaxis, chromodacryorrhea, rough fur, swelling of the nasal region, salivation, abdominal distention, diarrhea, paleness, external abodminal staining, lacrimation, hematuria, decreased body-weight gain, and body-weight loss. Peritonitis was seen in IP-dosed rats. In PO-dosed rats, signs of toxicity were: rapid or shallow breathing, sedation, loose and/or bloody feces, nasal discharge, dehydration of skin, chromodacryorrhea, and areas of hair loss. Additional acute toxicity studies showed that there was no difference in toxicity of mitoxantrone when administered each of the two vehicles (physiological saline and acetate-buffered formulation).

Single - dose IV toxicity, Rat:

No. of animals: 15/sex/dose level.

Dose levels: 0, 0.03, 0.1, 0.3, 1.0, 3.0 mg/kg IV.

Signs of toxicity: Body weight loss, decreased weight gain, epitaxis, rough fur, paleness, hematuria, diarrhea, hypothermia, and hair loss. No drug-related signs were present of doses of 1.0 mg/kg or lower.

Blood Chemistry:

These consisted of elevated cholesterol, triglyceride, urea nitrogen and alpha globulin fractions. Decreases in alkaline phosphatase, total protein, albumin and gamma globulin fractions were present. Generally, these finding occurred at doses of and 1.0 mg/kg.

Hematology:

Leukocytopenia, erythropenia (including decreases in hematocrit, hemoglobin, and erythrocyte count) were noted.

Gross and Microscopic Pathology:

Increase in organ weights of kidneys, liver and heart were observed at the 3.0 and 1.0 mg/kg dose levels. Microscopically, hydropic degeneration of proximal tubular epithelium, fibrotic change of glomeruli, and proliferation of intestinal tissue were observed in kidneys. Liver changes consisted of increases in basophilic granules and fatty degeneration. Changes consisted of vaculation of myocardial fibers accompanies by interstitial accumulation of inflammatory cells. Decreases in thymus and spleen weights were associated microscopically with depletion of lymphocytes in these organs. These changes were accompanied by decreases in myeloid cellularity bone marrow in rats given 0.3 mg/kg or higher.

Dog, single-dose IV toxicity (Beagle dogs).

1. 5 dogs/dose, 0.25, 0.5, 1.0, 4.0 or 40 mg/kg, 8 day observation period. No control group.

2. 2 dogs/dose level/sex, 0. (saline), 0.187.5, 0.25, 0.375, 0.5, or 1.0 mg/kg, 60-67 days observation period.

Signs of toxicity: diarrhea, decreased activity, weakness, emesis, salivation, hypothermia, decreased body weight.

Hematology: Erythropenia, leukopenia, and thromocytopenia.

Gross pathology: Pulmonary edema.

Histopathology: Bone marrow hypocellularity, lymphocytic depletion, GI tract damage, hemorrhage of lung, and hepatic congestion.

Lethal IV dose of Mitoxantrone in dogs was considered to be 0.5 mg/kg.

Monkey, single-dose IV toxicity: Two separate studies were conducted.

1. 1/dose level: 1.5, 3.0, 6.0, 12 or 60 mg/kg; 8 days observation period.

2. 2/dose level/sex; 0(saline), 0.25 0.5, or 1.0 mg/kg, 64-65 days observation period.

Signs of toxicity: Inactivity, clonic conclusions, shallow respiration, epitaxis, emesis, diarrhea, decreased body weight, hypothermia.

Hematology: Erythropenia, leukopenia.

Postmortem - Gross observation: Pulmonary edema, weight decrease in lymphoid organs.

Postmortem - Microscopic observation Similar to that were found in dog study.

The lowest single lethal IV dose was considered to be 1.0 mg/kg in monkeys.

Summary of mitoxantrone acute toxicity (IV) as follow:

Species	Days Observation	Lowest Lethal Dose (mg/kg)	Highest Non-Lethal Dose (mg/kg)
Mouse	21	9.8	7.8
Rat		3.5	2.9
		4.0	1.0
			6.0
			1.0
			0.375
			0.5

Overall, the dog is the most sensitive to the acute toxic effects of mitoxantrone.

In rats, dogs and monkeys, the predominant drug-related findings were gastroenteropathy and decreased cellularity of bone marrow. However, reversal of and recovery from toxic effects were obtained among survivors. In rats, kidney is also a target organ-effect of mitoxantrone. Cardiac changes were noted in rats surviving 35-56 days after dosing.

Subchronic multiple-dose toxicity studies.

1. Rat, daily IV administration for one month:

   10/sex/dose level; 0.003, 0.01, 0.03, 0.1, 0.3 mg/kg. All rats given 0.3 mg/kg/day died between 11th and 25th days of study; all others survived the dosing period. Toxic finding were similar to these seen in acute IV rat studies. Slight changes in cardiac tissue were seen by light microscopy given 0.1 or 0.3 mg/kg.

2. Dog, IV daily, X5:

   Beagle dogs; one/dose level; 0.1, 0.2, 0.4 or 0.8 mg/kg/day X5; 1-6 days observation period. No control in this study. All dogs survived the 5-day treatment phase but each dog at 0.8, 0.4 and 0.2 mg/kg/day was sacrificed moribund.

   Signs of toxicity included: emesis, bloody diarrhea, salivation, lethargy, hypothermia, body weight loss. Erthropenia, leukopenia and lymphopenia were observed. Transient increases in urea nitrogen and alkaline phosphatase were noted. Drug-related findings included enterocolitis, lymphadenitis, visceral congestion, bone marrow hypocellularity and aplasia of myeloid and erythroid cells.

3. Dog, daily IV for 14 days:

   2/sex/dose; 0(saline), 0.05, 0.1, 0.2; 21 days observation period.

   Only two females (one each of dose levels of 0.05 and 0.1 mg/kg/day) survived the treatment and observation periods. Toxic finding were confined mainly to drug-related effects on gastrointestinal and hematopoietic systems. Based on grass and microscopic examination, no morphologic changes indicative of anthracycline cardiomyopathy were observed in the myocardium.

4. Dog, 5 days, IV plus 9 days recovery, 3 cycles. Three dogs/sex/dose level; 0 (saline), 0.025, 0.05, 0.1 or 0.2 mg/kg/day; 16-32 observation period.

   Doses of 0.1 and 0.2 mg/kg were lethal. Signs of toxicity were gastroenteropathy and myelosuppressions. Microscopically, depletion of myeloid and erythroid cells in bone marrow, lymphocytic depletion, and generalized hemorrhaging of the heart, skeletal muscle, lymphoid organs, GI tract, lungs, kidneys and urinary bladder were noted. All males had degenerative lesions of the spermatogenic epithelium.

5. Monkey, IV daily, X5:

   One/dose level/day X5; No control; 0.3, 0.6, 1.2 or 2.4 mg/kg/day; 6 day observation period. The monkey receiving 2.4 mg/kg/day died. All other survived. Signs of toxicity were related to the GI tract. Depressed hematocrit, hemoglobin, RBC and WBC were observed. Drug-related gross and microscopic findings included visceral congestion and bone marrow hypocellularity.

6. Monkey, IV daily, X14:

   Three/sex/dose/day X 14; 0.05, 0.2 or 0.8 mg/kg/day; 21 day observation period. Only those receiving 0.05 mg/kg/day survived the 21-day observation period. Toxic signs again were associated with effects on the GI tract and bone marrow. Microscopically, myocardia and GI hemorrhage, decreased myeloid elements in bone marrow, and depletion of lymphoid elements in spleen, lymph nodes and tonsils were observed.

7. Monkey, I.V. once every 21 days, 2 cycles:

   5/sex/dose group; control (0.8% NaCl and 0.2% sodium metabisulfite), 0.5, 1.0 and 1.5 mg/kg; 1.64mg/kg (doxorubicin only). 9-week and 3-week observation period for mitoxantrone and doxorubicin, respectively. Only monkeys receiving 0.5 mg/kg survived the observation period. Effects that may indicate cardiac impairment were present in 4 out of 15 animals that died prior to schedule sacrifice. These were fluid in the peritoneal cavity and usually in the pleural space. There were no changes in EKG's or blood pressure that could relate to mitoxantrone or adriamycin administration. No microscopic examination was performed. In animals dying or sacrificed moribund, the results suggested a severe effect on bone marrow.

Chronic IV toxicity studies:

1. Rat IV administration once every 21 days for 12 months.
   39 rats/sex/dose, IV doses of 0.03, 0.3, 0.6, and 0.9 mg/kg; Phase I (6-month duration with 15 rats/sex/dose level) and Phase 2 (10-month duration, with 18 rats/sex/dose level) and Phase 3 (12-month duration plus 3-month recovery with 6 rats/sex/dose level).

   a. 6-month phase:

      All males given 0.6 or 0.9 mg/kg died during the 6-month phase. All but 3 females given 0.9 mg/kg died. Deaths also occurred among

females given 0.6 mg/kg. Eight deaths occurred among males receiving 0.3 mg/kg. Renal damage was among survivor at dose levels 0.3 mg/kg and above. Signs of toxicity wee similar to those described in one-month IV study and were associated with general physical deterioration. The alterations in hemogram were noted in animals given 0.3 mg/kg. The hematopoietic suppression noted generally in rats given 0.3 mg/kg or greater. At 0.03 mg/kg dose level, there were no drug-related toxic effect other than decreased food intake among males. Focal myocarditis was seen in rats found dead or sacrificed moribund.

b. 10 month phase with recovery:

The animals surviving through the 6-month phase were continued for a total of 4 additional months. All females at 0.6 and 0.9 mg/kg died. In addition, 2/24 females and 15/18 males died at 0.3 mg/kg dose level. Signs of toxicity were similar as previously mentioned. Serm chemistry changes included elevated SGOT, LDH, serum cholesterol, globulin, TG, phospholipid, and inorganic phosphorus among females given 0.3 mg/kg. Renal dysfunction and myelosuppression continued to be observed. In addition, swelling of hepatocytes was detected in most females given 0.3 mg/kg. Chronic myocarditis was observed. During recovery (3 months after 14th dose), there was no evidence of reversal of renal damage and lymphocytic depletion. There were no apparent treatment-related changes in surviving animals given 0.03 mg/kg.

c. 12-month phase with recovery:

Three remaining males and 5/10 females given 0.3 mg/kg and 1/12 males given 0.03 mg died. During the 3-month recovery period 4/4 females at the 0.3 mg/kg level died. Proteinuria was noted among males at 0.03 mg/kg. Again, at this level, blood chemistry parameters also altered. Males given 0.03 mg/kg, showed evidence of renal changes. Principal findings again were associated with the kidney lymphoid tissue and bone marrow.

2. Dog, 30-week IV toxicity:

The purpose of this study was to investigate the chronic toxicity of mitoxantrone and to compare the effects of this drug with the effects of doxorubicin in beagle dogs using an intermittent dosing schedule. The doses, i.e. 0.125 or 0.25 mg/kg of mitoxantrone or 1.64 mg/kg doxorubicin

were chosen. Sponsor stated that those doses produced similar patterns of leukopenia and recovery without producing life-threatening myelosuppression. Mortality in doxorubicin-treated dogs was greater than in mitoxantrone-treated dogs. The rate of recovery from the myelosuppressive effects seen in mitoxantrone-treated dogs was slower than that seen in doxorubicin-treated dogs. Therefore, mitoxantrone was considered more severe myelosuppressive than doxorubicin. Electrocardiographic changes and hypotension were noted in doxorubicin-treated dogs. In contrast to the progressive cardiomyopathy observed in the series of biopsy sample from doxorubicin-treated dogs, there were no lesions indicative of progressive cardiomyopathy in dog given mitoxantrone. However, electron microscopic examination did show dilation of the sarcoplasmic reticulum.

3. Monkey, 44-week IV toxicity:

Doses for the monkey study were the same as those selected for the dog study. Mortality in monkeys receiving doxorubicin was greater than in monkeys treated with mitoxantrone. The rate of recovery from the myelosuppresive effects seen in mitoxantrone-treated monkeys was faster than that seen in doxorubicin-treated monkeys. Again, mitoxantrone was considered more severe myelosuppressive than doxorubicin. ECG changes were noted in 1/10 doxorubicin-treated monkeys. Progressively decreasing blood pressure was noted in 8/10 doxorubicin-treated monkeys. Light microscopic examination, pale myocytes and vacuolated myocytes were observed in both drug-treated monkeys. The incidence and severity of these changes were greater in monkeys given doxorubicin. Electron microscopy of heart samples revealed myofibrillar loss and dilation of the sarco plasmic reticulum. In monkeys given mitoxantrone, myocytes exhibiting myofibrill loss but featured indicative of regeneration and active protein synthesis. In monkeys given doxorubicin, other pale myocytes showed signs of irreversible damage.

4. Rabbit, 21-week IV toxicity. Single dose every week X 15. 12/sex/dose level: 0.125, 0.25 (Mitoxantrone); 1.64 (Doxorubicin).

The first study was sponsored by the NCI. SPF New Zealand white rabbit were treated with dihydroxydiacetate form of mitoxantrone 0.8 mg/kg/week or adriamycin 2.0 mg/kg/week. "From this data, one can conclude that a dose of 0.8 mg/kg/week NSC-299195 was more toxic than ADR and not severe as adriamycin groups but resulted cardiac toxicity in rabbits." Commented by Dr. Lee-Ham (see IND 16,332 review, 8/27/81). Dr. Richman recommended that a comparative study of cardiotoxicity be conducted with the clinically employed compound.

The second study was performed by the sponsor. Dose selected for mitoxantrone (0.125 or 0.25) and doxorubicin (1.64 mg/kg/wk) were lower

than those in the NCI study. Because of deterioting physical condition and increased incidence of mortality, surviving rabbit receiving doxorubicin were sacrificed one week after 12 doses; surviving rabbits receiving mitoxantrone wer sacrificed 7 weeks after 15 doses. Morphologic cardiac changes were observed in both treated groups. EKG changes and hypotension were noted in doxorubicin-treated group. Nephrotoxicity were observed in both drug-treated animals. The absence of a leukopenic effect is in contrast to results in other species.

5. Dog IV toxicity, intermittent dosing after doxorubicin treatment. Three males and three females per group. The purpose of this study is to assess the effect of added mitoxicity treatment in dogs pretreated with cardiomyopathic doses of doxorubicin. Result from phase II (ongoing) of group 3 (4 doses of doxorubicin followed by mitoxantrone and group 4 (4 doses of doxorubicin followed by doxorubicin). There were 5 deaths during phase II. Four dogs were treated with 7-10 doses of doxorubicin (group 4) and one was treated with 4 doses of doxorubicin and 3 doses of mitoxantrone (group 3). One in group 3 and 3 in group 4 developed clinical signs suggestive of cardiac failure. EOG changes, hypotension were noted. Result may suggest that a trend of cardiac involvment in the morbidity was present in group 3. Final analysis of the experiment could not be drawn until a complete report of data is submitted.

Mutagenicity.

Microbial Mutogenicity:

Mitoxantrone was assayed at concentration of 0.1 to 1000 ug/plate and 10 to 500 ug/plate with and without rat liver microsomal activation using Salmonella typhimurium strains TA 1535, 1537, 1538, 98,000 and E. Coli strain WP-2 uvrA. Each study also employed positive and negative controls.

Result: The results indicated that mitoxantrone was positive in these systems.

Unscheduled DNA synthesis: This study was assessed using primary rat hypocytes in culture. Concentrations ranging from 0.0001 to 2.0 mg/ml were used. Positive and negative controls were also employed. Incorporation of radiolabeled thymidine was used as measure of unscheduled DNA synthesis. The results indicated that mitroxantrone caused DNA damage in this sytem.

Sister chromatid exchange:

Chinese Hamster Ovary cells were exposed to mitoxantrone at concentrations ranging from 0.31 to 10 ng/ml without metabolic activation and from 0.03 to 10 ng/ml with metabolic activation (S-9 mix). Positive and negative controls were included.

Result: Mitoxantrone produced a dose-dependent increase in the frequency of SCE's in CHO cells with and without metabolic activation.

Mouse Lymphoma Test:

Mutagenic potential of this drug was evalutated in the L5178Y TK+/- mouse lymphoma test system. Concentration of 0.0003 to 0.0015 mg/ml without metabolic activation and 0.03 to 0.15 ug/ml with activation were used.

Result: All concentrations caused a greater mutant frequencies than that of the negative control. Mitoxantrone produced a positive response in this system.

Cell Transformation:

Cell transformation of $C_3H/10T$ 1/2 mammalian cells in vitro were evaluated. Mitoxantrone (0.031 to 0.5 mg/ml) and doxorubicin (0.94 to 15.0 ng/ml) did not produce a statistically significant difference in frequency of transformed foci between treated and negative controls.

Chromosomal Abberation: Bone marrow cells in metaphase were examined microscopically for evidence of chromosome damage. IP doses of 0.5, 1.0 or 2.0 mg/kg/day X 5; or IV doses of 0.3 mg/kg on day 0 or day 21 were used.

Result: At doses of 1.0 and 2.0 mg/kg, multiple aberrations were seen. Twenty-one days after IV 0.3 mg/kg, no significant clastogenic effects occur in bone marrow.

Dominant Lethal Test:

Three groups of 10 male rats were given daily IP doses of 0.5, 1.0, or 2.0 mg/kg for 5 days. All rats in the 2.0 mg/kg dose group died during 4 weeks of mating. Females were sacrificed on day 11 to 15 days of pregnancy. The fertility of males given 2.0 mg/kg decreased. When pregnancy did occur, no effects on implantation or embryo survival were seen. No effects on fertility, implantation, or embryo survival were seen in the group given 0.5 or 1.0 mg/kg.

Reproduction:

Segmen I, rat, reproductive function: (Elm Farm Laboratories, Suffolk, England, Charles River CD strain: 28 rats/group; dose levels: 0.0033, 0.01, 0.03 mg/kg. IV administration to males of the Fo generation for 71 days prior to pairing, continued throughout the mating period and until termination after pregnancy of the Fo females had been confirmed. Females of the Fo generation were treated 15 days prior to pairing, continued throughout the mating period and until necropsy at day 21 post coitum or after weaning at day 25 post partum.

Result: No death occurred in treated animals. High dosage group female (0.03 mg/kg) showed a reduction in body weight gain during gestation. The regularity of oestrus, and mating performance and fertility were similar in all groups. In females, killed on day 21 of gestation, ovulation, implantation, survival, growth and development in utero showed no treatment-related effects. Litter size at birth was reduced in females receiving 0.03 mg/kg but subsequent viability was unaffected and post-natal growth, development and behavior showed no treatment-related effects. Absolute and relative weights of epididymides showed a slight dosage-related reduction for Fo males.

Segment II Teratology.

Rat: Mitoxantron was administered IV to 22 mated females rats per group daily on day 6-15 of pregnancy at dose level of 0.05, 0.1 or 0.2 mg/kg. Control females received normal saline.

Result: All animal survived except one dam in the high dose group. A significantly lower body weight gain was observed in the drug-treated groups and these changes were found to be dose related. The death of one dam was related most probably with the lesion found in urinary system. The fetal body weight per litter was significantly lower in the high and intermediate dose groups. Other reproduction parameters (resorptions, nidations, mean corpora Lutea) appeared unaffected by treatment. Two fetuses with severe abnormalities were found in the low dose group, however, these abnormalities were not observed in the intermediate and high dose groups. All skeletal findings can be classified either common variant or minor anomalies. Retarded development of the fetal kidney, as indicated by absence or small size of the renal papilla, occurred with greater frequency in drug treated groups.

Rabbit: Mitoxantrone was administered IV to 18 artifically inseminated rabbits per group on day 6-18 of pregnancy at dose levels of 0.01, 0.025, or 0.05 mg/kg/day. There were 3 spontaneous deaths resulting from respiratory infection. Increased incidences of premature delivery occurred in treated groups (5/17, 2/14, 4/16 and 1/16 for the high, intermediate, low and control groups, respectively).

Ear lesions along the injection sites were noted in the treated animals. There were no statistically significant differences in reproductive data between the treated and control groups. Five fetuses with major malformations were seen (2 in intermediant and control groups and one in the high dose group).

Carcinogenicity.

Mouse.

Study One: Oncogenic potential of mitoxantrone after IV administration to mice every 21 days for 2 years. The present report provides an summary of finding after approximately 10 months. Dose levels 0.0, 0.1, 0.2, 0.4 mg/kg/21 days.

Number of mice: 60/sex/dose level. Charles River CD-1 mice were used. The gross and microscopic changes that were observed are considered spontaneous occurrences. Study is still ongoing.

Study Two: Dose levels: 0, 0, 0.01, 0.03, 0.06 mg/kg/21 days for 5 months.

Number of mice: 60 dose/sex. At day 161, 4 mice were found dead. There appears to be no treatment related finding after the IV administration for 5 months at dose level up to 0.06 mg/kg/21 days.

Rat.

Dose Levels. 0, 0.01, 0.03, 0.1 mg/kg. Number of animals - 60-70/sex/dose level. The present report summarizes the finding after 21 months. There appears to be a higher incidence of auditory sebaceous gland (Zymbal's gland) neoplasms in males than would historically be expected. They have occurred in the control, the intermediate and high dose groups. There is an increased mortality rate in the high dose group. Final conclusion could not be made until the study is completed.

Irritation Study.

Topical Toxicity.

Rats.

Mitoxantrone was administered in a single application to the abreaded skin of the rats in doses of 100, 250, 500, 1000 or 2000 mg/kg.

Result: Red material around eyes, nose, facial and abdominal area, inactivity, sedation, unkemptness, soft and discolored feces, shallow and rapid breathing, blue skin eschar formation, loss of body weight. Blue colored kidneys were revealed in one rat (1000mg/kg). LD_{50} was 1640mg/kg. Mitoxantrone administered by dermal application to the abroded skin was readily absorbed and lethal.

Rabbit.

Dose levels: 125, 250, 500, 1500, 2000 mg/kg.

Result: All rabbits in 250-2000 mg/kg were dead except one female (1500 mg/kg) survived for 14 days. Animals treated with 125 mg/kg survived until day 14 except one male. Pale blue discoloration of sclera was noted. Body weight loss were noted in treated rabbits. Signs of toxic effect included sedation, decreased muscle tone and inactivity. The compound is absorbed by the skin and is lethal. Precaution should be taken to avoid skin contact.

Occular Irritation.

Rabbit.

In rabbits, mitoxantrone bulk powderor in 0.8% w/v sodium chloride and 0.2% w/v sodium metobisulfite irritated the eye. Swelling, discharge, reddening of the conjunctiva, and damage to the cornea and iris were seen in bulk powder form. Less irritation was noted in parental solution.

Dermal Sensitization.

Guinea Pig.

Guinea Pig received 0.1 ml intradermal injections of 0.1% mitoxantrone or doxorubicin. Topical application of filter paper saturated with 0.5% mitoxantrone and doxorubicin was given on day 7. On day 28, animals were challenged at new sites using the same procedure as for topical application. There were no toxic signs or mortality in this study.

Combination IV toxicity.

Dog.

Thirty-two beagle dogs (12 males and 11 females) were used to assess effects of mitoxantrone in combination with other agents. The dogs were distributed into 17 treatment groups in 4 separate experiments. In summary, mitoxatrone given to dogs at 0.3 mg/kg IV in combination with either cisplatinum, cytosine arabinoside, mitomycin C, prednisone, vincristine or vinblastine was not tolerated and resulted in mortality. In combination with cyclophosphamide and 5-FU (only at 15 mg/kg) or with methothrexate and 5FU (18 mg/kg), mitoxantrone is tolerated with signs of myelosuppression.

Heparin Activity.

When solutions of heparin and mitoxantrone were combined, the formation of a dark blue precipitate and reductions in heparin activity were concentration related.

Summary and Evaluation.

This NDA is for Mitoxantrone (Novantrone) for injection, on antineoplastic drug used for the treatment of breast cancer, including locally advanced or metastatic disease.

It is supplied as an aqueous solution containing mitoxantrone hydrochloride equivalent to 2 mg/ml mitoxantrone free base, with sodium chloride, sodium acetate, and acetic acid as inactive ingredient.

Mitoxantrone showed antitumor activity in several mouse tumor systems, such as P388 Leukemia, L1210 Leukemia, B-16 melanoma (IP implanted), Colon tumor 26, CD8F1 mammary carcinoma and murine colon carcinoma. It has also been shown to be moderately effective in P388/adria and P388 MAMSA. In conventional mouse test systems with IP implanted tumors, its activity is greater than or comparable to other antineoplastic agents.

It has been shown to be an inhibitor of RNA and DNA synthesis. It binds to RNA and DNA. It induceds nuclear aberration in cell cultures of a human colon carcinoma line. Its cytocidal effect on both actively dividing and non-dividing-cultured human WiDr and WI-38 cells, indicating it may be a cell cycle non-specific agent. Its mechanism of action has not been determined.

The therapeutic index of mitoxontrone is eight to fifteen times greater than that of doxorubicin against IP implanted leukemia as stated in page 81 of volume 1 (report 7). However, in report 1, a different therapeutic index was obtained (TI=34 and 66 for mitoxantrone and Adriamycin, respectively).

Mitoxantrone produced the greatest cytotoxicity in cells treated while in G1 or G2 phases although it was also effective against cell in S phase or mitosis depending upon concentrations. Therefore, it may indicate that mitoxantrone is not cell cycle phase-specific.

Pharmacokinetics: Mitoxantrone demonstrates rapid plasma clearance, a long elimination half-life and extensive tissue distribution in both animal and human following IV doses.

In rats, dogs, monkeys and humans given single dose of ^{14}C-mitoxantrone, radioactivity concentrations disappear rapidly from both plasma and whole blood during the first 2 hours after dosing, concentration decrease slowly thereafter. It disappears from plasma/serum with a mutiphasic (bi or tri-phasic) pattern characterized by a very rapid plasma clearance and low renal, hepatobiliary and metabolic clearance. Total radioactivity has linear, sex-independent, and dose-independent characteristics in rats. Elimination half-life of mitoxantrone in rat and human is about 12 days. A rapid initial distribution phase followed by a relatively slow elimination phase orphases.

Excretion: In rats, dogs and monkeys, 10 days after ^{14}C-mitoxantrone, 63 to 83% of administered radioactivity is accounted for in the excreta; 80-90% of the recovered radioactivity is excreted in the feces and 10-20% is excreted in the urine. There is a discrepancy between (1) elimination half-life in rat is 12 days in report 33 and, (2) 83% of administered ^{14}C-mitoxantrone is excreted in the feces and urine after 10 days in report 29.

Bile is the major excretory route in rats. Little radioactivity was found in the bile of rats given radiolabeled mitoxantrone orally, indicating the poor absorption through oral administration.

Mitoxantrone is rapidly and extensively distributed into the organs of rats, dogs, monkeys and humans. In animals, radioactivity is highest in bile, gallbladder (except rats), liver, spleen and kidney. Slight amounts of radioactivity (50 mg equivalents/g) were seen in spinal cord, CSF, brain, cornea aqueous humor, vitreous humor, testes (rat) and temur (dog). In pregnant rats dosed with 0.5 mg/kg of ^{14}C-mitoxantrone on day 18 of gestation, uptake by the placenta is noted. 0.4% of the total concentration of drug in the placenta was present in the fetus. 7 ng/ml of mitoxantrone was found in the amniotic fluid one hour after dosing. It appeared to be similar in man in terms of its tissue distribution.

Studies indicated that metabolism in all four species (rat, dog, monkey, and human) is excreted as unchanged, and two polor metabolites and their glumonide conjugates. The two metabolites isolated from human urine were identified as the mono and dicarboxylic. Uptake of melanoma were low relative to other tissues of the mouse. Sponsor suggested that melanoma tissue containing a large necrotic core contribute to this low concentration of radioactivity in tumor tissue.

Toxicology

Mice: Single dose toxicity studies.

LD10 and LD50 are as follow (mg/kg):

Route	Sex	LD10	LD50
IV	M	7.8	11.3
	F	7.1	9.7
IP	M	7.1	16.5
	F	2.3	19.7
IV[a]	combined		12.2
IV[b]	M		10.4
	F		10.6

[a]0.8% w/v NaCl and 0.2% w/v sodium metabisulfite.

[b]Acetate-buffered formation (final clinical formation).

There is a difference in final clinical formation between in P381, Vol. 26 and P143 Vol. 1.

LD50 values in both vehicle were comparable. Toxic signs observed were reduced activity, listlessness, cyanosis, salivation, prostration piloerection and palpebralptosis, paleness, rough fur, lacrimation and hypothermia.

Rats LD50

Route	Sex	LD10(ng/kg)	LD50(mg/kg)
IV	M	3.5	4.8
	F	3.6	5.2
IP	M	6.2	8.0
	F	9.9	11.7
PO	M	422	682
	F	474	721

Signs of toxicity: Epistaxis, rough fur, salivation, swelling of nasal region, diarrhea, paleness, lacrimation, hematuria, weight-loss, abdominal distension and staining. Hair loss and hind-limb edema were seen in IV-treated rats. LD50 values of mitoxantrone in clinical formulation are comparable to that in physicological saline.

Single I.V. toxicity:

Lethal dose: 3.0 mg/kg.

Highest non-toxic dose: 0.1 mg/kg.

Animals developed previously-mentioned signs of toxicity. ↓Serum alkaline phosphate, ↓protein, ↓albumin and ↓gamma globulin, ↑Serum cholesterol, ↑TG, ↑urea nitrogen, ↑globulin, leukocytopenia, lymphopenia, erythropenia, ↓platelet count were noted.

Bone marrow and kidney were primary target organs. Kidney changes were characterized by hydropic degeneration of proximal tubular epithelium, fibrotic changes of glomeruli and proliferation of interstital tissue. Some liver lesions were observed at 3.0 mg/kg dose group. Heart change were confined to 3.0 mg/kg dose group. Focal myocarditis was noted.

Dog, Single IV Toxicity:

The lowest single lethal dose was 0.5 mg/kg. Signs of toxicity were related to effects on the GI tract such as emesis, diarrhea, decreased food consumption and body weight. Other signs such as inactivity, hypothermia, leukopenia, erythopenia, thrombocytopenia were also noted. Bone marrow hypocellularity and lymphocytic depletion of lymphoid organs. High dose also caused hepatic congestion.

Monkey, Single IV Toxicity:

The lowest lethal dose was 1.0 mg/kg. Signs of toxicity were similar to that of dogs.

Multiple-dose subchronic and chronic studies.

Rat, daily IV X 30:

Maximum tolerated dose in rat is 0.1 mg/kg. Signs of toxic effect were similar to that of acute toxicity study. Changes in cardiac tissue were seen in rats given 0.3 mg/kg by light and electron microscopic evaluation. Bone marrow and kidney were targets of organ.

Dog, daily IV X 5:

Clinical signs of toxicity included emesis, blood diarrhea, calivation, lethargy, hyperthermin weight loss. Maximun tolerated dose - 0.2 mg/kg. Bone marrow involvement and GI toxicity were observed. None were seen in the dog receiving 0.1 mg/kg/day.

Daily IV X 14:

MTD is 0.05 mg/kg. Drug related effects were again similar to that of 5 daily studies e.g. GI and hematopoietic systems at all dose levels. Cardiac changes were observed. However, no morphologic changes indicative of anthracycline cardiomyopathy were observed.

5 daily IV + 9 day recovery, 3 cycles:

MTD is 0.05 mg/kg. Clinical signs of toxicity were similar to those in previous repeated dose studies, i.e. GI toxicity and myelosuppressor. All males had degenerative lesion of the spermatogenic epithelium.

Monkey: MTD for daily IV X 5 is 1.2 mg/kg.
MTD for daily IV X 14 is 0.05 mg/kg.
MTD for single dose/21 days X 2, is 0.5 mg/kg.
GI toxicity and myelosuppression were the major toxic effects.

[illegible] studies in rats, dogs, and monkeys were designed to [illegible]ity of mitoxantrone with special attention given to the [illegible] the cardiomypathy. Mitoxantrone was given IV to dogs [illegible]weeks at dose levels of 0.125 and 0.25 mg/kg; [illegible]inistered at dose level of 1.64 /kg. These doses [illegible] without life-threatening myelosuppression and [illegible]cluded that mitoxantrone was evaluated for [illegible]myelosuppressive condition than [illegible] of cardiomyopathy nor irreversible [illegible] receiving mitoxantrone. However, [illegible].125 and 0.25 vs. 1.64 mg/kg, about

6-fold difference). Myelosuppressive toxicity limits the dose levels to be comparable to that of doxorubicin. Therefore, I do not consider this comparative cardiotoxicity data for evaluating the potential cardiotoxicity conclusive. It may indicate that cardiomyopathy was not observed when mitoxantrone was given to animal at 1/6 to 1/7 doses level of doxorubicin. This is comparable to its recommended dosage: 12 mg/m^2 in mitoxantrone as compared to 70 mg/m^2 in doxorubicin.

Rabbit given 0.8/kg/week of mitoxantrone died after 3 to 8 weeks. Besides its renal toxicity, cardiac effects were also observed. Sponsor suggested that these effects were secondary to generalized organ toxicity involving the kidney, liver and bone marrow. However, this could not rule the possibility of its direct effect on cardiac tissue of rabbit. The absence of a leukopenic effect is in contract to results in other species. In another rabbit study, the cardiac toxicities between mitoxantrone (0.125 or 0.25 mg/kg) and doxorubicin (1.64 mg/kg) were compared. Cardiac changes were accompanied by renal toxicity in mitoxantrone-treated rabbit. The cardiac effects seen in mitoxantrone-treated animals were not typical of progressive anthracycline-like cardiomyopathy.

Genetic Toxicology.

Mitoxantrone caused point mutations. It caused increases in unscheduled DNA synthesis (DNA damage). It also induced sister chromatid exchanges in CHO cells. It caused chromosomal aberrations in vivo cytogenic study by 5 daily IP administration. The incidence of chromosomal damage by IV every 21 days for 2 doses resembled that found in controls. It did not induce cell transformation in mammalian cells in vitro. It did not cause a dominant lethal effect in rate by IP administration of mitoxantrone daily for 5 days.

Reproduction.

For Fo males there were dose-related decreases in epididymol weights. High dosage group females (0.03 mg/kg/day) showed a reduction in body weight gain during gestation. In females killed on day 21 of gestation, ovulation, implantation, survival, growth and development in utero showed no treatment-related effects. Sponsor should also sacrificed half of females on day 13 of gestation. The dems should be examined for number and distribution of embryos in each uterine horn, presence of empty implantation sites and embryo undergoing resorption. Embryo death due to abnormal condition in the uterus should also be examined. Sponsor stated that doses (highest: 0.03 mg/kg/day) were based on results of daily IV mitoxantrone (X 30) study. However, MTD was 0.1 mg/kg day as indicated at P162 of Vol. 1 (Table 28). Litter size at birth was reduced in females receiving 0.03 mg/kg.

Teratology.

Rat: Lower body weight gain was observed and were found to be dose related. The fetal body weight was significant lower in the high and intermediate dose groups. Retarded development of fetal kidney occurred with greater frequency

in drug treated groups. Sponsor considered both findings to be due to maternal toxicity. Although the amount of mitoxantrone transfer from the placenta to the fetus or ammiotic fluid is very small (sponsor stated negligible). Accumulation of mitoxantrone by daily injection of mitoxantrone for 10 days may exceed a level which caused the retarded development of fetal kidney. Therefore, I don't think it is fair to rule out this possibility. Kidney is a target organ effect of mitoxantrone in rats.

Rabbit: Increased incidence of premature delivery occurred in treated groups. Other parameters appeared to be normal.

Carcinogenicity studies: Study have not been completed.

Other Toxicity Studies: Mitoxantrone is absorbed through the skin causing motality in rabbits and rats. It also caused ocular irritation. Mitoxantrone and heparin should not be administered together in the same solution due to its precipitate formation.

Package Insert:

The carinogenesis, mutagenesis section is adequate. The pregnancy category is given as B - We recommended the modified D category.

Following items should be corrected or modified in Vol. 26 (labeling).

(1) P381, sodium metabisufite should be included in its final clinical formulation.

(2) P383, "In pregnant rats the placenta is an effective barrier" should be modified due to small amount of mitoxantrone did cross the placenta.

(3) P385, "In vitro studies using the clinical dosing regimen showed no residual mutagenic effect" should be added or modified because chromosomal damage did occurr when drugs were administered IP daily for 5 days in rats.

Recommendation:

Due to its accumulation potential, retarded development of fetal kidney and decrease in fetal body weight in teratology studies, I will recommend it is approvable if the modified pregnancy category D will be required in the label. Package insert (labeling) should be modified as indicated in "Package-insert" section.

Ching-Long Joseph Sun, Ph.D.
November 23, 1984

CC: Orig IND 19,297
HFN-150/ Div. File
HFN-150/ Dr. Sun, 11/23/84
HFN-150/ R. Podliska
F/T by db, 5/6/85
Wang # 0900B

Pharmacological Review of Supplement NDA 19297
Date of submission:10/18/85
Date of Review Completed: 2/28/86

NOV 29 1986

SPONSOR: Lederle

DRUG: Novantrone

DRUG CATEGORY: Antineoplastic Agent

MATERIALS REVIEWED:
Carcinogenecity Study
Rat.

1. No. of Studies:one.
2. Name of Laboratory: American Cyanamide Co. Wilbur G. Malsolm Toxicology Lab.
3. Strain:Crl:COBS^{R}CDR(SD), Charles River
4. No of animals:70/Sex/controls, 60/sex/drug-treated group
5. Doses:0,0,0.01,0.03,0.1 mg/kg,i.v. every 21 days
6. Basis for dose selection stated: yes, based on 10 months study (i.v. every 21 days) that the cummulative dose threshold at which myelosuppresion is irreversible and death ensues was estimated to be approximately 3.5 mg/kg.
7. Interim sacrifice: no, sacrificed when death and moribound during study and at end of study.
8. Total duaration : 25 months.
9. Week/site for first tumor:
   At 28 wk, lymphoma were found in bone marrow, liver, lyph nodes, urinary bladder.
   External auditory canal
   0(62 wk), 0.01 (102 wk), 0.03 (78 wk), 0.1(53wk)
   Fibroma:
   Female
   0(57 wk), 0.1mg/kg(98 wks)
10. No. of alive at termination:

	0	0	0.01	0.3	0.1 mg/kg
24 months:					
male	19*	26*	25*	15*	1*
female	48	36	26*	30	17*
25 months:					
male	16*	22*	21*	12*	0*
female	45	29*	17*	21*	6*

*: mortality> 50%

11. Statistical methods used: Trend test (Tarone procedure), Heterogenecity (Peto et al.).
Tumor data : as shown on next page.

Q: NDA 19-29

Sex	Males							Females						
Group Number[b]	1	2	3	4	5	P Value[c] Hetero- geneity	Trend	1	2	3	4	5	P Value Hetero- geneity	Trend
Liver (N)	69	70	60	60	59			69	70	58	59	60		
Carcinoma Hepatocellular	1	0	1	1	0	*	*	0	0	0	0	0	*	*
Cholangiocarcinoma	0	0	0	0	0	*	*	0	0	0	1	0	*	*
Pancreas (N)	68	68	59	60	59			69	70	57	58	60		
Islet Cell Tumor	18	8	6	5	2	.1592	.93446	7	5	6	5	5	.7114	.16798
Thymus/Lymph Node (N)	61	56	52	56	46			57	51	39	47	49		
Leukemia Granulocytic	1	1	1	0	2	*	*	0	0	2	1	0	*	*
Lymphoma	1	0	5	1	1	.0380	.50017	0	0	2	0	0	.1344	.46322
Thymoma	0	1	1	0	0	*	*	0	1	0	0	0	*	*
Spleen (N)	70	70	60	60	59			69	70	57	59	60		
Leukemia, Granulocytic	1	1	1	0	1	*	*	0	0	1	1	0	*	*
Lymphoma	1	0	3	3	0	.0708	.36380	0	0	1	0	0	.1743	.46407
Adrenal Glands (N)	67	70	59	60	59			70	69	57	60	60		
Carcinoma, Cortex	1	0	1	0	1	*	*	2	1	1	0	0	*	*
Medullary tumor	5	8	3	8	3	.0354	.04217	1	2	0	2	1	.5808	.26270
Testes (N)	70	70	60	58	59									
Interstitial Cell tumor	2	1	2	1	1	.7475	.24794	NA	NA	NA	NA	NA		
Pituitary Gland (N)	65	66	57	60	57			69	69	57	59	58		
Adenoma	35	37	28	28	9	.0115	.99804	58	58	51	48	45	.7155	.64773
Bone Marrow (N)	69	70	60	59	59			69	70	58	59	59		
Lymphoma	1	0	2	3	0	.1147	.33698	0	0	1	0	0	.1870	.46417
Leukemia, Granulocytic	0	1	1	0	1	*	*	0	0	1	1	0	*	*
Skin (N)	69	69	60	60	59			69	70	58	60	60		
External Auditory Canal	2	0	1	4	8	.0005	.00006	0	1	0	1	8	.0001	.00005
Carcinoma Squamous	0	0	0	1	0	*	*	0	0	0	0	3	*	*
Fibrosarcoma	0	0	0	0	2	*	*	0	0	0	1	2	*	*
Fibroma	7	4	2	2	4	.2694	.51908	1	0	0	0	6	.0001	.00006
Lipoma	1	1	1	1	0	.9529	.56812	0	3	4	1	1	.2470	.54523
Mammary Gland (N)	30	33	26	33	34			67	64	52	55	55		
Adenocarcinoma	0	1	1	0	1	.2949	.19070	6	5	8	1	3	.0703	.85506
Carcinoma	0	0	0	0	0	*	*	2	3	0	0	1	.3041	.86756
Adenoma	0	0	1	0	0	.4235	.37712	19	8	7	9	4	.5509	.84466
Fibroma	1	0	0	0	0	*	*	3	0	0	0	1	*	*
Fibroadenoma	0	1	0	0	0	.7283	.83499	16	22	16	14	18	.5921	.13247
Lipoma	0	0	0	0	0	*	*	1	0	0	1	1	*	*
Thyroid (N)	69	69	60	60	59			69	70	58	59	60		
Adenoma	4	3	3	3	2	.9974	.49134	3	2	3	1	0	.4216	.84135
Carcinoma	1	0	0	1	1	*	*	0	1	1	0	0	*	*
Adenoma Parafollicular	5	4	2	1	0	.5281	.91709	15	5	7	6	2	.4133	.91005
Carcinoma, Parafollicular	0	0	0	2	1	*	*	0	1	0	0	0	*	*
Parathyroid (N)	67	67	60	59	60			69	70	58	58	60		
Adenoma	1	0	0	0	1	*	*	0	1	0	1	0	*	*

Sex	Males							Females						
Group Number[b]	1	2	3	4	5	P Value[c] Hetero-geneity	P Value[c] Trend	1	2	3	4	5	P Value Hetero-geneity	P Value Trend
Liver (N)	69	70	60	60	59			69	70	58	59	60		
Carcinoma Hepatocellular	1	0	1	1	0	*	*	0	0	0	0	0	*	*
Cholangiocarcinoma	0	0	0	0	0	*	*	0	0	0	1	0	*	*
Pancreas (N)	68	68	59	60	59			69	70	57	58	60		
Islet Cell Tumor	18	8	6	5	2	.1592	.93446	7	5	6	5	5	.7114	.16798
Thymus/Lymph Node (N)	61	56	52	56	46			57	51	39	47	49		
Leukemia Granulocytic	1	1	1	0	2	*	*	0	0	2	1	0	*	*
Lymphoma	1	0	5	1	1	.0380	.50017	0	0	2	0	0	.1344	.46322
Thymoma	0	1	1	0	0	*	*	0	1	0	0	0	*	*
Spleen (N)	70	70	60	60	59			69	70	57	59	60		
Leukemia, Granulocytic	1	1	1	0	1	*	*	0	0	1	1	0	*	*
Lymphoma	1	0	3	3	0	.0708	.36380	0	0	1	0	0	.1743	.46407
Adrenal Glands (N)	67	70	59	60	59			70	69	57	60	60		
Carcinoma, Cortex	1	0	1	0	1	*	*	2	1	1	0	0	*	*
Medullary tumor	5	8	3	8	3	.0354	.04217	1	2	0	2	1	.5808	.26270
Testes (N)	70	70	60	58	59									
Interstitial Cell tumor	2	1	2	1	1	.7475	.24794	NA	NA	NA	NA	NA		
Pituitary Gland (N)	65	66	57	60	57			69	69	57	59	58		
Adenoma	35	37	28	28	9	.0115	.99804	58	58	51	48	45	.7155	.64773
Bone Marrow (N)	69	70	60	59	59			69	70	58	59	59		
Lymphoma	1	0	2	3	0	.1147	.33698	0	0	1	0	0	.1870	.46417
Leukemia, Granulocytic	0	1	1	0	1	*	*	0	0	1	1	0	*	*
Skin (N)	69	69	60	60	59			69	70	58	60	60		
External Auditory Canal	2	0	1	4	8	.0005	.00006	0	1	0	1	8	.0001	.00005
Carcinoma Squamous	0	0	0	1	0	*	*	0	0	0	0	3	*	*
Fibrosarcoma	0	0	0	0	2	*	*	0	0	0	1	2	*	*
Fibroma	7	4	2	2	4	.2694	.51908	1	0	0	0	6	.0001	.00006
Lipoma	1	1	1	1	0	.9529	.56812	0	3	4	1	1	.2470	.54523
Mammary Gland (N)	30	33	26	33	34			67	64	52	55	55		
Adenocarcinoma	0	1	1	0	1	.2949	.19070	6	5	8	1	3	.0703	.85506
Carcinoma	0	0	0	0	0	*	*	2	3	0	0	1	.3041	.86756
Adenoma	0	0	1	0	0	.4235	.37712	19	8	7	9	4	.5509	.84466
Fibroma	1	0	0	0	0	*	*	3	0	0	0	1	*	*
Fibroadenoma	0	1	0	0	0	.7283	.83499	16	22	16	14	18	.5921	.13247
Lipoma	0	0	0	0	0	*	*	1	0	0	1	1	*	*
Thyroid (N)	69	69	60	60	59			59	70	58	59	60		
Adenoma	4	3	3	3	2	.9974	.49134	3	2	3	1	0	.4216	.84135
Carcinoma	1	0	0	1	1	*	*	0	1	1	0	0	*	*
Adenoma Parafollicular	5	4	2	1	0	.5281	.91709	15	5	7	6	2	.4133	.91005
Carcinoma, Parafollicular	0	0	0	2	1	*	*	0	1	0	0	0	*	*
Parathyroid (N)	67	67	60	59	60			69	70	58	58	60		
Adenoma	1	0	0	0	1	*	*	0	1	0	1	0	*	*

Mouse carcinogenecity study:

1. No. of studies: 2.
2. Name of laboratory: Wilbur G. Malcolm Lab.
3. Strain: CD-1, Charles River.
4. No. of animals: 60/Sex/group.
5. Doses: Ist study: 0,0.1,0.2,0.4 mg/kg
   2nd study: 0.0.01,0.03,0.06 mg/kg , i. v. every 21 days.
6. Basis for dose selection: yes , based on 60-days study(i.v. every 21 days).
7. Interim sacrifice: no, sacrificed when death or moribound during study and at end of study.
8. Total duration: 25 months.
9. No. of alive at termination:

lst study:

at 96 wks

	0	0	0.1	0.2	0.4
male	26	28	27	21	2
female	29	25	29	29	4

at 24 months

male	21	25	22	16	3
female	23	16	20	16	2

at 18 months survival rates were larger than 50 % among all groups.

2nd study:

at 25 months

	0	0.01	0.03	0.06
male	40%	48%	53%	35%
female	42%	32%	40%	35%

at 96 wks

between 60-70 % among all groups

10. Statistiscal methods used:heteogeneity and Trend tests(Tarone procedure) with respect to mortality patterns or the incidence patterns of palpable masses.

11. Tumor and non-tumor data fr each tissue: no data were submitted concerning the histopathological finding of the studies.
12. Week/site for first tumor: no data submitted for this respect.

Results:

Rats.

Body weight and food consumption: Low and mid dose animals were similar to controls. In high-dose males, body weight decline more rapidly than in controls. In high-dose females, body weight was slightly less than controls starting at month 18.

Sponsor stated that the most probable cause of death in males was glomerulnephritis. Moartality in females was associated with pituitary adinoma, often acconmpanies by glomerulonephritis.

Clinical pathology: Increases in UN and creatinine were observed in the females groups (low and mid-dose).

Organ weight: Relative weight increase in heart, lungs, pituitary gland and spleen were seen in the males. Increase in liver,kidney, heart and brain were seen in the females.

Histopathologic findings: Tumors were found in the skin of external ear canal of both sex. Fibroma were also reported in the female. Both incidences of tumor were statistically significant.

Mice.

Body wieght, food consumption: Treated animals were similar to the saline treated proup.

Physical finding and opthalamic examinations were similar amon all groups.

Hematology: There were some flutuational changes in some parameters. The sponsor stated that these changes were not toxicologically significant.

Urine chemistry: Changes between controls and treated animals were not remarkable.

Histopathology:
Non-neoplatic finding: Renals changes, subcutaneous edema and left atrial thrombosis are represented in high incidence among all study groups. The glomerulonephritis was not seen.
Neoplastic finding: The sponsor did not submit any detail reports on tumor incidence because only either single occurrence, or a few in goups were observed.

Conclusion: The carcinogenic study (rats) indicated a very high incidence of mortality, particularly in the males. Serveral factors could contribute to this cause. One of them probably be due to aging of the rats. Different strain of rat may be more appropriate for long term study, such as Fisher 344. Mortality at end of 24 months (104 wks) were more than 50 % in all male groups and in controls and mid-dose female groups. However, at end of 18 months, the mortalities among all controls and drug-treated groups were better than 50 %. According to the materials (formats) submitted, tumors in external ear canal and liproma were observed.

Mortalities among mice were also high. At end of 96 wks, suvival rates were between 60 and 70 % in the second study. Survival rates were around the vicinity of 50% except in high-dose groups and mid-dose male group in the first study. There was no complete carcinogenic data submitted in this NDA. The sponsor should be informed that complete report should be submitted in order to evaluate its carcinogenic potential. Complete detail formats of carcinogenic study should follow the recomendations from the Division of Biometrics.

Pharmacology: Activity of metabolite

Metabolites of mitoxantrone which were identified as mono- and dicarbosylica acid derivates were tested for activity against human colon carcinoma cells in vitro using a clonogenic assay. Both metabolites were inactive at concentrations ranging from 0.125 to 4 ug/ml for CL283981 and from 0.3 to 5 ug/ml for CL285049. Cl283981 was also inactive when tested for antitumor activity against the P388 leukemia in BDF1 mice.

Pharmacokinetis: The data indicated that when dogs were given mitoxantrone following doxorubicin treatment, both serum and blood radioactivity concentrations were higher in doxorubicin pretreated dog following the first dose of ^{14}C-mitoxantrone. But this interfernce disappeared with succeeding doses.

Toxicology:

Cardiotoxicity: Dog,i.v., intermittent dosing after doxorubicin treatment.

The purpose of this study was to detemine whether mitoxantrone exacerbated doxorubicin-induced cardiomyopathy Intermittent dosing with mitoxantrone (0.25 mg/kg/d,x6) began 7 or 19 weeks after the last dose of doxorubicin (1.64 mg/kg, x6). The sponor suggested that mitoxantrone was not additive to doxorubicin induced cardiomyopathy.However, there was an increase in degree of myocardial lesions as indicated at page 1838 that degree of lesion was increased from grade 1 (female received 4 doses of doxorubicin) to grade 2 (male recieved 4 doses of Dororubicin and 3 doses of MTX).

Genetic toxicology:

Mouse. For the cytogenetic study, five male and five female mice form the control and 0.1 mg/kg, and the surviving four male and six female mice from 0.2 mg/kg group and one male and one female from the high-dose (0.4 mg/kg) group were included in the evaluation. The animals were sacrificed 21 days after last dose of mitoxantrone (every 21 days for 2 years in carcinogenic study). Mitoxantrone given to mice at doses of 0.1 to 0.4 mg/kg every 3 weekd for 2 years did not produce chromosomal damage as indicated in this submission.

Rat. As in mouse study, femoral bone marrow cells in metaphase were examined microscopicallly for evidence of chromosomal acerrations. Rats were given 0.01, 0.3 or 0.1 mg/kg, every 21 days for 2 years. Five male and femals from the control, low-, and intermediated-dose groups, seven females from the high-dose group were selected for evaluation. Again, there was no increase in the incidence of chromosomal aberrations 21 days after mitoxantrone. However, chromosomal aberration did occur in higher dose levels as indicated in the previous reports (REports 137 and 138).

LABELING: (201.57 pakage insert)
Clinical pharmacology:

Mechanism of action: It appered that a description of the biochemical and/or physiological mode of action was not clearly stated. Details regarding this respect should be elaborated.
Since this is a anthrocycline-like drug, animal toxicology/pharmacology should be mentioned in the section, or if not appropriate, in a seperate section.

Warning scetion:
A "preganancy category D " should be inserted. A statement of modified pregnancy category D should be stated in " Carcinogenesis, mutagenesis, impairment of fertility' section.

Carcinogenesis, mutagenesis, impairment of fertility:
Rat carcinogenic study was invalid due to its high mortalities among treated and control groups particularly in the males. Fibroma and tumor at external auditory canal were found.. In addition, no detail and complete mouse study were submitted and sponsor also considered that the studies were only preliminary. Therefore, that statement concerning its carcinogesis should be modified.The sponsor should submit detail carcinogenecity study in order to complete evaluation.
Chromosomal aberration did occur following i.p or i.v. administration of novantrone. Althouhg the sponsor stated that life time (2 years) studies in mice and rats using the clinical dosing regimen(i.v. every 21 days)showed no residual chromosomal aberration. However, the highest dose level used in those study were only a fraction of the recommended clinical dosage. Thus, chromosomal aberration following novantrone should be mentioned and statement of no chromosomal aberration in life-time rat and mouse studies should be modified to avoid misleading.

Based on the nature of its pharmacologic, biochemical, or cytotoxic action , it may be expected to present significant risk of fetal abnormalities if used in pregnancy women. Pregnancy category C should change to modified pregnancy D (see original review).

Nursing mother:
Since the drug is associated with serious adverse reaction, the labeling shall state: It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for seriou adverse reaction in nursing infant from _____, a decision should be made whether to discontinue, nursing or to discontinue the drug,.....".

TOXICOLOGY: Additional published papers conderning its toxicology were submitted.

Hematopoietic toxicity (performed by Okuewick et al, 1985. report 11). The suthors reported that hematopoitic toxicity of MTX was more severe than that of DX. The suthors stated that in humans the equipotent myelosuppreseive doses of MTX vs DX were in a ratio of 1/4.

Cardiac toxicity:
Dog and monkey (performed by the sponsor). In previous submission, the sponsor stated that myocardial lesions in the dogs or myocardiopathy in the monkeys were observed in DX-treated but not in MTX-treated groups. However, the studies were not conducted at equal dose basis (3 mg/kg of MTX vs 16.4 mg/kg in monkey and 0.25 of MTX vs 1.64 mg/kg of DX in dog). Thus, the conclusion of this compatative studies could not be reached.

Mouse and guinea pig. (performed by Perkins et al. 1984, report 12, Adria Lab.). The author reported similar finding for MTX and DX. At 2 mg/kg dose level, MTX caused more incidence of cardiac damage (86%) than that of DX (22%). The data also indicated that 2 mg/kg of MTX appeared to cause the same incidence of cardiac damage as that of 4 mg/kg of DX. Therefore, theses data suggested that MTX may have a spectrum of myocardial activity similar to that of DX and may be more toxic in mice.

Hamster. (performed by Dantchev et al, 1984). Although MTX was classified by the authors as less toxic than DX in term of tis myocardial alteration, the dosages used were not equal (3 mg/kg of DX vs 1 mg/kg of MTX).

Dog and rabbit. (performed by Grieshaber of NCI, 1984). A caoparative study of DX and MTX in rabbit indicated that MTX was not as cardiatoxic as DX when injected for 12-wk period. When MTX was administered for more than 12 wk, cardiomyopathy was seen in 5 of 6 rabbits. Dosages used in this study were 48 $mg/m^2/wk$ for MTX and 24 $mg/m^2/wk$ for DX. MTX used in this study was anthracenedione diacetate.

In conclusion, although the sponsor provided more data (including some published papers) trying to indicate that a less cardiac toxicity of MTX than that of DX. Most of studies were compared at different dosages (lower dose of MTX was used in most of cases) except at rabbit study where higher dose of MTX than that of DX was chosen. As reported by the authors and the sponsor that apparent cardiac toxicity of MTX did exixt. Since no conclusive comparison of cardica toxicity between MTX and Dx was reached, clinical data shall play an important role in its risk-benefit assessment of its potential usage in treating cancer patients.

RECOMMENDATION:

We would recommend that it is approvable based on pharmacological and toxicological points of view. However, sponsor should also submit detail from carcinogenetic study (submitted in the format should follow the recommendation from the Division of Biometrics) in order to perform complete evaluation. Pakage insert should be modified as indicated in the " pakage-insert " section.

Ching+Long Joseph Sun Ph. D.

cc:
Orig. NDA 19-297A ✓
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