# Disposition of Mitoxantrone in Cancer Patients[1]

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## ABSTRACT

We have used a highly sensitive high-performance liquid chromatographic assay to evaluate the pharmacokinetics and tissue disposition of mitoxantrone, an investigational anthracene derivative which has shown significant activity during Phase II clinical trials in the treatment of metastatic breast cancer, unfavorable histology non-Hodgkin's lymphoma, and acute leukemia. Mitoxantrone (12 mg/sq m over 30 to 35 min in 250 ml of dextrose 5% in water) and $^{14}$C-labeled mitoxantrone (specific activity, 8.85 $\mu$Ci/mg) were administered to eight patients who had advanced soft tissue cancers. The plasma disappearance of mitoxantrone concentrations measured by high-performance liquid chromatography was best described by a three-compartment model with a mean $t^\alpha$ of 0.1 h, a $t^\beta$ of 1.1 h, and a $t^\gamma$ of 42.6 h. The mean apparent $V_c$ was 12.2 liters/sq m, while the mean $V_d$ was 1875 liters/sq m. The mean plasma clearance was 0.57 liters/min/sq m, and the mean renal clearance was 45 ml/min/sq m. Only 6.5% of the total mitoxantrone dose was excreted in the urine as unchanged drug over 5 days. The mean recovery of $^{14}$C-labeled material in feces over 5 days was 18.3% of the administered dose. Thirty-five days after mitoxantrone administration to a patient who died of progressive kidney cancer, approximately 15% of the $^{14}$C dose could be accounted for in seven major organs. We conclude that mitoxantrone appears to distribute into a deep tissue compartment from which it is slowly released. These data provide a pharmacological rationale for use of mitoxantrone on an intermittent dosing schedule.

## INTRODUCTION

Mitoxantrone, or 1,4-dihydroxy-5,8-bis{{{2-[(2-hydroxyethyl)-amino]ethyl}amino}}-9, 10-anthracenedione dihydrochloride (NSC 301739) is an investigational anthracene derivative which has shown significant activity during Phase II clinical trials in the treatment of metastatic breast cancer, unfavorable histology non-Hodgkin's lymphoma, and acute leukemia (6, 9, 18, 20, 22). Mitoxantrone has been very well tolerated by the cancer patient, causing a very low incidence of nausea, vomiting, and alopecia, and virtually no phlebitis or soft tissue damage in instances of extravasation (3, 21). Its acute dose-limiting toxicities have proven rapidly reversible leukopenia and to a lesser extent thrombocytopenia (3, 21). In animal studies, mitoxantrone has shown no evidence of cardiotoxicity; however, accumulating data from Phase II clinical trials suggest that chronic mitoxantrone dosing is associated with a low but significant incidence of a cardiomyopathy (2, 17, 19). We (11, 12) and others (10, 13) have developed HPLC[3] assays for mitoxantrone in order to study potential relationships between mitoxantrone disposition, clinical toxicity, and antitumor activity (4).

## MATERIALS AND METHODS

**Patient Selection.** Each adult male or female patient entered into this study had to meet the following requirements: (a) histologically proven malignant disease in advanced stage; (b) disease unresponsive to all other potentially curative forms of therapy; (c) recovery from the toxicity of prior radiation treatment or chemotherapy; (d) life expectancy of at least 1 month; (e) normal renal and hepatic function; and (f) prior total Adriamycin dose less than 350 mg/sq m and no clinical evidence of cardiac disease.

**Radiolabeled Mitoxantrone.** Mitoxantrone labeled with radioactive carbon was supplied by the Medical Research Division of American Cyanamid Co., Pearl River, NY. The radiopurity of the compound was 96%; the location of the $^{14}$C was as shown in the structural formula of mitoxantrone in Chart 1. The specific activity of the compound was 8.85 $\mu$Ci/mg of mitoxantrone. Each dose was individually packaged in sealed ampuls which contained 2 mg of mitoxantrone (as the free base) per ml of solution. The volume of each ampul was 10.5 ml. In addition to the drug, the sterile solution contained sodium metabisulfite (0.20% w/v) and sodium chloride (0.8% w/v) and water for injection.

**Drug Administration, Sample Collection, and Preparation.** Each patient was administered a single i.v. dose of mitoxantrone, 12 mg/sq m, over 30 to 35 min in 250 ml of dextrose 5% in water. Blood samples were obtained from the opposite arm at −30, −10, −5, 0 (end infusion), 1, 3, 5, 10, 15, 20, 30, and 45 min, and 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, 96, and 120 h after the termination of mitoxantrone infusion. Blood samples were drawn into heparinized tubes, immediately placed on ice, and then quickly processed to separate plasma and RBC and WBC which were then frozen at −20° C for analysis. Total urinary and fecal outputs were collected for up to 5 days postdosing. Tumor tissue was biopsied in 3 patients, bone marrow aspirates were taken from 2 patients, and organ specimens were obtained from one patient at autopsy in order to determine mitoxantrone concentrations (as measured by $^{14}$C equivalents).

**Analytical Procedures.** Mitoxantrone concentrations in plasma and urine were determined by both total radioactivity (i.e., mitoxantrone equivalents) and HPLC. Tumor tissue, fecal material, blood-formed elements, and saliva were analyzed for $^{14}$C content only. A recently reported HPLC assay for mitoxantrone (12) was used to quantitate parent compound in all plasma and urine samples. A detailed description of this assay appears below.

**HPLC Assay Materials.** Mitoxantrone reference standards, obtained from Lederle Laboratories, American Cyanamid Co., were dissolved in methanol and stored at −80° C. Organic solvents were distilled in glass (Burdick and Jackson Co., Muskegon, MI) and filtered through a 0.45-

[3] The abbreviations used are: HPLC, high-performance liquid chromatography; FE, formed elements.
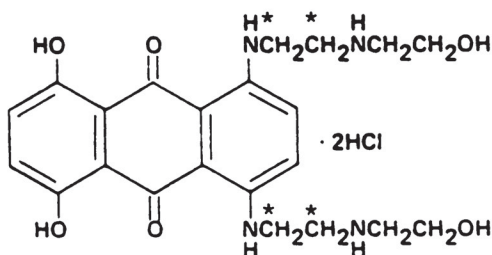
Chart 1. Chemical structure of mitoxantrone (1,4-dihydroxy-5,8-bis||2-[(2-hydroxyethyl)amino]ethyl|amino||-9,10-anthracenedione dihydrochloride). *, positions of ¹⁴C radiolabels.

$\mu$m Fluoropore filter (Millipore Corp., Bedford, MA) prior to use. Aqueous solvents for HPLC use were filtered through a 0.45-$\mu$m cellulose acetate filter prior to use. Ammonium acetate (HPLC grade) was obtained from Fisher Scientific Co. (Fair Lawn, NY). L-Ascorbic acid was obtained from Grand Island Biological Co. (Grand Island, NY). Human albumin and $\gamma$-globulin were obtained from Sigma Chemical Co. (St. Louis, MO), and 3 N methanolic-HCl was obtained from Supelco (Bellefonte, PA).

**HPLC Sample Cleanup Procedure.** A VAC-ELUT system equipped with BOND-ELUT 1-ml $C_{18}$ cartridges (Analytichem International, Harbor City, CA) was used for sample cleanup. One- to 2-ml plasma samples were passed through a cartridge which was sequentially preconditioned by washing with 10 ml of methanol and 5 ml of $H_2O$. After the plasma had passed through the cartridge, it was washed with 5 ml of $H_2O$, and 300 $\mu$l of 0.5 N methanolic-HCl were used to elute the drug. The eluate was collected, vortexed, and kept at $-20°$ C for HPLC analysis.

**HPLC Apparatus.** HPLC was performed with an apparatus consisting of a Model 660 solvent programmer, 2 Model 6000A solvent delivery systems, a Model U6K injector, a Model 440 detector (Waters Associates, Milford, MA), and a Model A-25 recorder (Varian, Palo Alto, CA). A Waters Associates $C_{18}$-$\mu$Bondapak (3.9-mm inside diameter x 30 cm) reversed-phase column preceded by a 7-cm x 2.1-mm (inside diameter) guard column packed with CO:PELL ODS (Whatman Inc., Clifton, NJ) was used for all analyses. The guard column packing was changed every 2 weeks or whenever there was significant back pressure buildup.

**HPLC Assay Chromatographic Conditions.** Mitoxantrone was eluted isocratically at ambient temperature with a solvent composition of 25% $CH_3CN$ and 75% ammonium acetate (0.2 M, pH 4.0) at a flow rate of 1.5 ml/min. Mitoxantrone was detected at 658 nm using a Waters Associates Model 440 fixed-wavelength detector.

**HPLC Assay Quantitation.** Quantitation of mitoxantrone was done by the external standard method of analysis. Plasma standard curves were obtained by plotting the resulting peak heights against the known concentration of standards added to the plasma samples.

**Tissue Preparation for Quantitation of ¹⁴C-Labeled Mitoxantrone Equivalents.** Plasma, urine, and saliva samples were prepared in a similar way. In each case, 0.5 ml of sample was added to 0.5 or 1 ml of solubilizer plus 10 ml of Aquasol, mixed, and counted in a scintillation counter for 10 min. Whole bone marrow, whole blood, and RBC (0.1 to 0.2 ml) were added to 0.5 ml of concentrated acetic acid in a scintillation vial and mixed until RBC were solubilized; 0.5 ml of hydrogen peroxide (30%) was added, and the solution was mixed well and heated to 60° C for 15 min until decolored. The samples were then cooled at room temperature, each added to 10 ml of Aquasol, and counted for 10 min in the scintillation counter. Each sample was then run in at least duplicate.

Two ml of water were added to each 1 g of fecal sample and sonicated. One ml of the fecal mixture was then added to 10 ml of solubilizer in a scintillation vial and heated at 60° C for 30 min. Then 0.1 ml of the digested material was added to 10-ml Ready-Solv-HP, mixed well, and counted for 10 min in the scintillation counter.

All samples were run in at least duplicate. Organ samples were weighed and oxidized using a Packard Tri-Carb Model B306 sample oxidizer. The ¹⁴$CO_2$ formed during oxidation was trapped in 4 ml of Carbosorb (Packard Instrument Co.). Twelve ml of Beta-Phase (West

Chem Co.) scintillation fluid were added. The resultant cocktail was counted on a Beckman LS100 liquid scintillation counter for 10 min. The data obtained were expressed as ng/g, wet weight, of tissue.

Each tumor biopsy was disaggregated into a single cell suspension. The cell suspension was then washed and counted. Aliquots of the cell suspension were added to a glass scintillation vial containing 10 ml of Aquasol, and were then counted for 10 min in a Beckman LS230 scintillation counter. All counts were corrected for background and counting efficiency. The data were expressed as ng/million cells.

The solubilizer used for these experiments was BTS-450 from Beckman, and the Aquasol was type NEF-934 from New England Nuclear. The Ready-Solv-HP was also obtained from Beckman. The scintillation counter was used at Channel B ¹⁴C with a gait of 260 and a preset error of 0.2%.

**Plasma Mitoxantrone Pharmacokinetic Analysis Techniques.** The mitoxantrone concentration *versus* time plasma data were fit to the multiexponential equation

$$C = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$$

using BMDP 3R, a nonlinear regression computer program. A weighting function of $1/y^2$ was used in order to fit the later time points. Initial parameter estimates are required for nonlinear regression and were obtained using CSTRIP. F-Tests were carried out to determine the number of compartments needed. Since there were insufficient data points during the infusion period to adequately define the area under this portion of the curve, for the pharmacokinetic analysis, the infusion data were adjusted to represent the equivalent of a bolus injection, using the mathematical equations described by Gibaldi and Perrier (7).

## RESULTS

**Patient Demography and Diagnosis.** Eight patients were enrolled into this study. The demographics and diagnostic characteristics of the patient population are presented in Table 1. There were 3 male and 5 female patients with a mean age of 57.9 years (range, 25 to 74). The mean body surface area was 1.61 sq m (range, 1.37 to 1.90), and the mean weight was 60.8 kg (range, 45.7 to 77.0). All patients enrolled in the study had advanced, soft-tissue primary disease (4 head and neck cancers, 2 renal cancers, 1 ovarian cancer, and 1 melanoma). None had primary bone involvement or leukemia, although one patient (Patient 2) had skeletal metastases. Prior to pharmacokinetic study, 3 patients (Patients 5, 6, and 7) had received 2, 2, and 12 doses of 12 mg mitoxantrone/sq m, respectively. These 3 patients were all receiving mitoxantrone once every 3 weeks. All other patients were studied during the first dose administration.

**Plasma and Blood Cellular Drug Concentrations.** The plasma mitoxantrone (HPLC) and [¹⁴C]mitoxantrone (expressed as mitoxantrone equivalents ng/ml) concentrations were obtained in 7 of the 8 patients. No plasma samples were taken from Patient 8 because of difficulty with adequate peripheral vein access. Plasma mitoxantrone and [¹⁴C]mitoxantrone concentrations decreased rapidly during the first 1 to 2 h after infusion completion. Thereafter, the decrease was slower. During the initial time period, plasma radioactivity concentrations were approximately equal to plasma mitoxantrone concentrations measured by HPLC. The decrease in plasma radioactivity concentrations was best described by dividing the patients into 2 groups. In Group 1 (Patients 1, 2, and 4), plasma radioactivity concentrations approximated plasma mitoxantrone concentrations for 5 to 20 min. At the later time periods, plasma radioactivity concentrations decreased less rapidly than plasma mitoxantrone concen-

Table 1
*Demographic and disease status characteristics of patients entered into pharmacokinetic study of mitoxantrone*

| Patient | Disease type | Site of metastasis | Height (cm) | Wt (kg) | BSA (Mxm) | Age (yr) | Sex/ race | Total dose (mg) | Prior mitox- antrone |
|---------|-------------|-------------------|-------------|---------|-----------|----------|-----------|-----------------|---------------------|
| 1 | Renal leiomyosarcoma | None | 156 | 51.5 | 1.42 | 58 | F/C[a] | 17 | No |
| 2 | Renal adenocarcinoma | Bone (multiple) | 168 | 49.8 | 1.37 | 40 | F/C | 16 | No |
| 3 | H and N squamous cell | Lung; submandibular lymph node | 176 | 73.5 | 1.90 | 74 | M/C | 23 | No |
| 4 | Ovarian adenocarcinoma | Cul-de-sac; supracla- vicular lymph node | 166 | 47.5 | 1.42 | 67 | F/C | 18 | No |
| 5 | Salivary gland adenocarci- noma | Lung | 172 | 72.7 | 1.85 | 70 | M/C* | 22 | Yes (2)[+] |
| 6 | Tongue squamous cell | Lung | 164 | 69.0 | 1.75 | 71 | M/C | 21 | Yes (2)[+] |
| 7 | Submandibular gland adeno- cystic carcinoma | Lung | 175 | 77.0 | 1.80 | 58 | F/C | 22 | Yes (12)[+] |
| 8 | Melanoma | Skin (multiple) | 154 | 45.7 | 1.40 | 25 | F/C | 18 | No |
| Mean | | | 168.8 | 60.8 | 1.61 | 57.9 | | 19.6 | |
| Minimum | | | 156 | 45.7 | 1.37 | 25 | | 16 | |
| Maximum | | | 172 | 77.0 | 1.9 | 74 | | 23 | |

[a] C, Caucasian of European ancestry; C*, Mexican; +, number of prior courses of mitoxantrone.
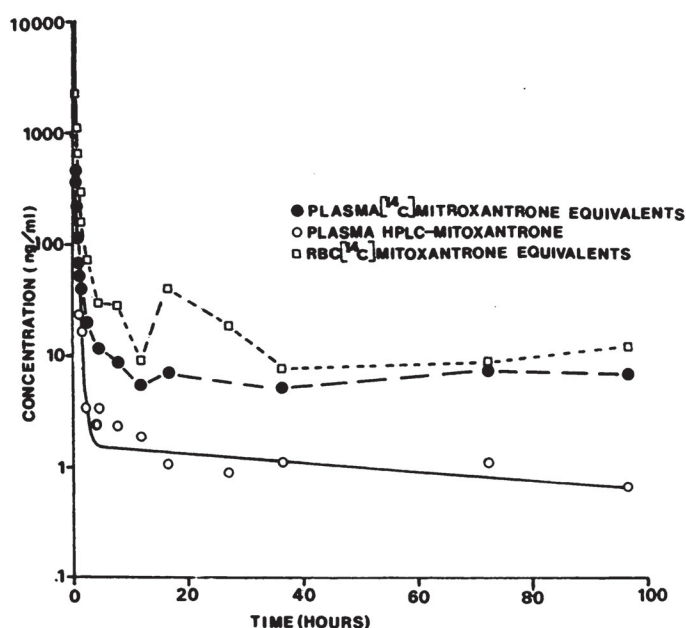


Chart 2. Concentration time data for mitoxantrone measured by HPLC and ^{14}C-radioactivity in plasma and in RBC. [14C]Mitoxantrone (specific activity, 8.85 μCi/mg) was administered i.v. over a 30-min period in a dose of 12 mg/sq m to Patient 4 (Tables 1 and 2).



Chart 3. Concentration time data for mitoxantrone measured by HPLC and ^{14}C-radioactivity in plasma and in RBC. [14C]Mitoxantrone (specific activity, 8.85 μCi/mg) was administered i.v. over a 30-min period in a dose of 12 mg/sq m to Patient 6 (Tables 1 and 2).
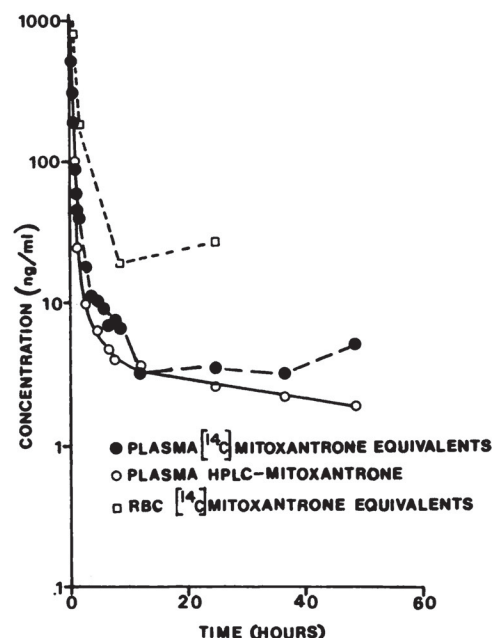
trations (*e.g.*, Chart 2). The difference persisted for the remainder of the study, approaching a 5-fold difference when the plasma mitoxantrone concentration had reached 2 ng/ml. In the other group (Group 2) of patients (Patients 3, 5, 6, and 7), the decline with time after administration of plasma radioactivity concentrations paralleled the decline of the plasma mitoxantrone concentrations with little difference between the 2 (*e.g.*, Chart 3).

The [14C]mitoxantrone content in blood FE was also determined following separation of plasma. The concentration of radioactivity in the FE was consistently greater than that in the plasma. The FE/plasma ratio of radioactivity ranged from 2/1 to 10/1 at various sampling times after mitoxantrone administration (*e.g.*, Charts 2 and 3).

**Plasma Pharmacokinetic Parameters.** In 5 of the 7 evaluable patients, the plasma disappearance of mitoxantrone concentra-

tions measured by HPLC were best described by a 3-compartment model. The mean pharmacokinetic parameters for these 5 patients are shown in Table 2. Because the data from the remaining 2 patients (Patients 4 and 5; Tables 1 and 2) were adequately described by a 2-compartment model, they have not been included in this analysis. In Patient 4, the terminal-phase (γ) plasma half-life of mitoxantrone was similar to that determined for Patients 1, 2, 3, 6, and 7, but her $t_{1/2}$ was an average of the α- and β-phases of the 3-compartment fit. In Patient 5, the plasma concentrations of mitoxantrone were below the sensitivity level of our HPLC assay (*i.e.*, <1 ng/ml plasma) within 7 h of drug administration. Thus, it was not possible to determine accurately a plasma terminal elimination phase in this patient.

In the 5 patients shown in Table 2, the initial phase of the decrease in plasma mitoxantrone concentrations was rapid

Table 2
*Individual pharmacokinetic parameters*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 12-mg/sq m dose | | | | | | | | | |
| Patient | $\alpha$ (h$^{-1}$) | $\beta$ (h$^{-1}$) | $\gamma$ (h$^{-1}$) | $t^\alpha$ (h) | $t^\beta$ (h) | $t$ (h) | $Cl_T$ (liters/ min/sq m) | $Cl_R$ (ml/ min/sq m) | $V_c$ (liters/ sq m) | $V_{d\gamma}$ (liters/sq m) |
| 1 | 8.7 | 0.67 | 0.03 | 0.078 | 1.04 | 20.8 | 0.94 | 85.8 | 9.7 | 1695 |
| 2 | 9.6 | 0.67 | 0.01 | 0.072 | 1.05 | 51.7 | 0.65 | 55.4 | 12.4 | 2930 |
| 3 | 8.5 | 0.72 | 0.01 | 0.082 | 0.95 | 69.3 | 0.30 | 18.5 | 3.9 | 1797 |
| 6 | 3.7 | 0.45 | 0.01 | 0.187 | 1.54 | 46.2 | 0.47 | 27.1 | 24.6 | 1878 |
| 7 | 6.2 | 0.93 | 0.028 | 0.112 | 0.75 | 25.0 | 0.50 | 38.6 | 10.3 | 1072 |
| Mean ± SD | 7.3 ± 2.4 | 0.69 ± 0.17 | 0.02 ± 0.01 | 0.106 ± 0.048 | 1.07 ± 0.29 | 42.6 ± 19.96 | 0.57 ± 0.24 | 45 ± 22 | 12.18 ± 7.62 | 1875 ± 670 |

Table 3
*Mean urinary recovery of mitoxantrone and [$^{14}$C]mitoxantrone*

| | | % of administered dose | | |
|---|---|---|---|---|
| Time (h) | No. of samples | [$^{14}$C]Mitoxantrone | No. of samples | Mitoxantrone |
| 0–4 | 6 | 2.9 | 6 | 3.7 |
| 4–8 | 6 | 1.2 | 6 | 1.2 |
| 8–16 | 5 | 0.6 | 5 | 0.4 |
| 16–24 | 5 | 0.6 | 5 | 0.5 |
| 0–24 | 7 | 5.6 | 7 | 5.9 |
| 24–48 | 7 | 1.4 | 6 | 0.5 |
| 48–72 | 7 | 1.0 | 7 | 0.3 |
| 72–96 | 7 | 0.8 | 6 | 0.3 |
| 96–120 | 4 | 0.9 | 4 | 0.2 |
| Mean total recovery (0 to 120 h) | | 10.1 | | 6.5 |
| Range | | 6.2–23.5 | | 5.2–7.9 |

Table 4
*Fecal [$^{14}$C]mitoxantrone recovery*

| Patient | No. of bowel movements | % of dose recovered (0–120 h) |
|---|---|---|
| 1 | 9 | 17.6 |
| 2 | 0 | 0 |
| 3 | 1 | 0 |
| 4 | 7 | 15.4 |
| 5 | 4 | 13.6 |
| 6 | 5 | 20.0 |
| 7 | 4 | 24.8 |
| 8 | 1 | 2.2 |
| Mean %[a] of administered dose (0–120 h) | | 18.3 |
| Range | | 13.6–24.8 |

[a] Includes only those patients who had more than one bowel movement.

Table 5
*Saliva [$^{14}$C]mitoxantrone concentrations in Patient 8*

| Time post infusion (h) | Concentration equivalents (ng/ml) |
|---|---|
| 0.25 | 3.8 |
| 0.50 | 5.2 |
| 0.75 | 8.5 |
| 1.00 | 2.5 |
| 2.00 | 4.3 |
| 3.25 | 3.3 |
| 4.25 | 6.1 |
| 8.00 | 7.5 |
| 12.00 | 11.4 |
| 22.50 | 5.6 |
| 72.00 | 5.5 |
| 96.00 | 4.0 |
| 120.00 | 3.6 |

Table 6
*[$^{14}$C]Mitoxantrone equivalents in biopsy and autopsy specimens*

| Patient | Tissue | Time after [$^{14}$C]mitoxantrone (h) | Concentration[a] equivalents (ng/liter × 10$^6$ cells) |
|---|---|---|---|
| 2 | Bone marrow | 6 | |
| | Whole | | 0.06 |
| | RBC only | | 0.05 |
| | WBC only | | 1.13 |
| 3 | Squamous cell, tumor nodule, neck | 5.75 | 0.06 |
| 4 | Adenocarcinoma, supraclavicular lymph node | 6 | 1.32 |
| 8 | Metastatic melanoma nodule, wrist | 22.75 | 0.03 |

[a] 1 × 10$^6$ cells = ~1 mg.

(mean $t_{1/2}$ = 0.1 h). The second phase ($t_{1/2}$) was somewhat longer with a mean half-life of 1.1 h. The mean terminal half-life ($t_{1/2}$) was 42.6 h. The mean apparent volume of the central compartment ($V_c$) was 12.2 liters/sq m, while the mean apparent volume of distribution ($V_{d\gamma}$) was 1875 liters/sq m. The mean plasma clearance ($Cl_T$) was 0.57 liters/min/sq m, and the mean renal clearance ($Cl_R$) was 45 ml/min/sq m, which represents 12% of the plasma clearance.

**Urinary Recovery of Mitoxantrone and [$^{14}$C]Mitoxantrone.** The mean urinary recoveries of mitoxantrone and [$^{14}$C]mitoxantrone at intervals of 4 to 120 h following drug administration are shown in Table 3. Only 6.5% (range, 5.2 to 7.9%) of the total mitoxantrone dose was recovered as unchanged drug over 5 days (120 h). The majority (90%) of drug was recovered during the first 24 h with the first 4 h contributing the largest quantity (62%). The HPLC chromatograms of patient urine samples showed consistently up to 3 more polar metabolite peaks than that which identified the parent compound.

Mean recovery of $^{14}$C-labeled material during the 120-h collection period was 10.1% (range, 6.2 to 23.5%) of the administered dose. Fifty-five % was recovered during the first 24 h. The recovery of $^{14}$C-labeled material for 7 of 8 patients (excluding Patient 2) ranged from 0.8- to 1.6-fold the parent compound (*i.e.*, mitoxantrone) recovery. Patient 2 excreted 23% of the administered dose as [$^{14}$C]mitoxantrone of which only 7.9% (as a percentage of dose administration) proved to be parent compound.

**Fecal Recovery of Mitoxantrone and [$^{14}$C]Mitoxantrone.** Feces were collected from 7 patients during the 5 days of study. Due to the health status and dietary restrictions of the patients, fecal output was variable, ranging from 0 to 9 collections over 120 h (Table 4). In those patients who had more than one bowel movement during the 5-day collection period, the mean percentage of recovery of $^{14}$C-labeled material was 18.3% (range, 13.6 to 24.8%) of the administered dose.

**[$^{14}$C]Mitoxantrone in Saliva.** Saliva was collected from patients to determine if radiolabeled drug was secreted via this

pathway. Only 1 to 3 samples were collected from most patients; however, Patient 8 provided several additional specimens throughout the 120-h collection period. Drug recoveries from saliva obtained from Patient 8 are shown in Table 5.

**Tissue Concentrations of [¹⁴C]Mitoxantrone Equivalents.** As shown in Table 6, tumor biopsies were obtained 5 to 22 h following [¹⁴C]mitoxantrone administration in 3 patients (Patients 3, 4, and 8). Only pg quantities of mitoxantrone equivalents per $10^6$ cells were recovered from a squamous cell tumor nodule (Patient 3) and a melanoma nodule (Patient 8) in 2 nonresponding patients. Of interest was the observation that metastatic tumor tissue from one of these patients (Patient 8) had proven extremely sensitive *in vitro* to mitoxantrone (*i.e.*, less than 1% survival of tumor colony-forming units at 10 ng/ml for a 1-h exposure) as measured by a human tumor clonogenic assay (14). A metastatic supraclavicular lymph node aspirated 6 h after mitoxantrone in a patient with ovarian cancer (Patient 4) revealed considerably higher amounts of [¹⁴C]mitoxantrone equivalents (*i.e.*, >1 ng/$10^6$ tumor cells). Although this patient was evaluated as a nonresponder to mitoxantrone, the lymph node disease did decrease in volume by greater than 25% during therapy.

A bone marrow aspirate obtained 6 h after mitoxantrone administration to a patient (Patient 2) with skeletal metastases secondary to a renal cell cancer showed approximately a 20-fold greater concentration of ¹⁴C-drug equivalents in the WBC *versus* RBC fraction (as separated by a Ficoll-Hypaque gradient) (Table 6). The ratio of whole marrow to plasma (adjusted for a hematocrit of 30%) was approximately 8/1. This patient experienced life-threatening leukopenia (*i.e.*, neutrophil count <500/cu mm) 10 days following mitoxantrone therapy.

Organ specimens were obtained from one patient (Patient 2) who died of progressive kidney cancer 35 days after mitoxantrone administration. Even at 35 days, the liver, pancreas, thyroid, spleen, and heart contained relatively high mitoxantrone equivalents per g of tissue (wet weight) (Chart 4). On the basis of [¹⁴C]mitoxantrone distribution per whole organ, the liver contained the highest amount of drug followed by the bone marrow, heart, lungs, spleen, kidney, and thyroid glands in that order (Chart 5). Adding the total amounts of mitoxantrone retained in each of these 7 organs as much as 15% of the administered dose could be accounted for in these tissues at 35 days.

## DISCUSSION

We have shown that the plasma disappearance of mitoxantrone measured by HPLC can be described in the majority of
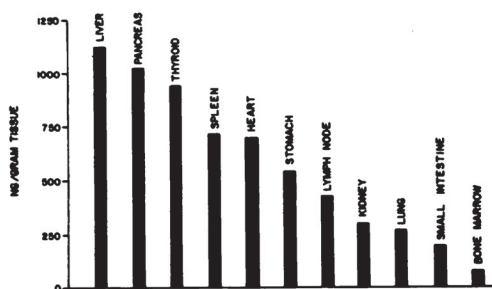
Chart 4. [¹⁴C]Mitoxantrone equivalents in ng per g of tissue (wet weight) obtained at autopsy 35 days after i.v. administration in a dose of 12 mg/sq m to Patient 2 (Tables 1 and 2).
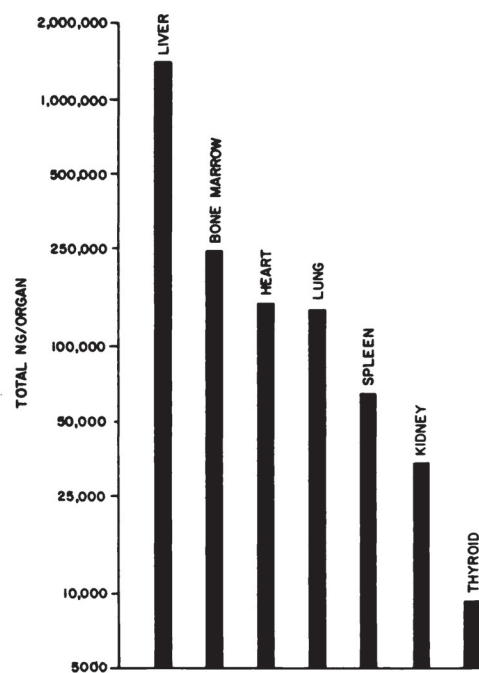
Chart 5. [¹⁴C]Mitoxantrone equivalents in ng per whole organ (wet weight) obtained at autopsy 35 days after i.v. administration in a dose of 12 mg/sq m in Patient 2 (Tables 1 and 2).

patients by a 3-compartment model with a prolonged terminal elimination phase half-life of approximately 43 h. Previous investigations have reported either shorter (8) or similar (15, 16) durations of this important pharmacokinetic parameter. Our highly sensitive HPLC assay (12) has allowed us to measure mitoxantrone plasma concentrations for up to 72 h after drug administration, and thus accurately determine the duration of the terminal elimination phase half-life. While the estimate of this half-life (*i.e.*, range of 20.8 to 69.3 h) is longer than that reported by others (8), it is likely that the true elimination half-life is much longer based on the body content of drug 35 days after dosing. These data provide a pharmacological rationale for the use of mitoxantrone on an intermittent dosing schedule.

Mitoxantrone appears to distribute into a deep tissue compartment from which it is slowly released as evidenced by its prolonged plasma terminal-phase half-life, extremely large volume of distribution ($V_D$), and the relatively large amount of mitoxantrone (>15% of administered dose) which appeared to be retained in autopsy tissues 35 days after dosing. Even though mitoxantrone (and/or mitoxantrone-related material) may persist in the body for prolonged periods, repeat dosing at 3-week intervals for as many as 12 courses had no noticeable effect on the calculated pharmacokinetics.

Considerable evidence exists to suggest that mitoxantrone undergoes extensive metabolism, probably in the liver. (*a*) The mean area under the plasma disappearance curve for ¹⁴C-labeled material was significantly greater than that determined by HPLC. However, in Patients 3, 5, 6, and 7 (Tables 1 and 2; Chart 3), the decline with time after administration of [¹⁴C]mitoxantrone plasma concentrations paralleled the decline of the plasma mitoxantrone concentrations measured by HPLC with little difference between them. It is possible that, in this group of patients, for unknown reasons, there was a lesser degree of metabolism

than occurred in Patients 1, 2, and 4. There was no evidence of liver dysfunction in any of these patients which could have explained differences in the pattern of mitoxantrone plasma clearance. (b) The recovery of ^{14}C-labeled material in the 5-day urine collections was significantly greater than that of the HPLC-measured parent compound. (c) Urine HPLC chromatograms revealed up to 3 polar metabolites, which appeared identical to those observed previously in rat bile.[4] (d) Ehninger et al. (5) have shown recently, using an isolated perfused rat liver model and thin-layer chromatography assay techniques, that mitoxantrone is actively metabolized with up to 3 more polar compounds appearing on the thin-layer plates. The identity and potential activity of these putative metabolites have not been determined due to their relatively small quantities and insensitivity of the gas chromatography-mass spectrometry techniques thus far applied to these studies.

The most important route of mitoxantrone elimination appears to be fecal. Total drug-related material recovered in urine plus stool averaged 28% of the administered dose in 5 days; 10.1% in the urine (6.5% as mitoxantrone and an additional 3.6% as ^{14}C-labeled material) and 18% in the feces. Because of the relatively low urinary excretion of mitoxantrone and its metabolites, it is unlikely that the standard drug dose must be reduced in the presence of compromised renal function. On the other hand, since the drug appears to be metabolized in the liver, future studies must be carried out to determine the effect of liver dysfunction on the disposition and toxicity of mitoxantrone. Indeed, Savaraj et al. (15) have shown that patients treated with mitoxantrone who had either abnormal liver function or a third space appeared to have significantly prolonged terminal-phase plasma half-lives and decreased rates of total body clearance of the parent compound.

The limited salivary secretion data obtained in this study show prolonged, but relatively low concentrations of mitoxantrone in saliva for up to 120 h after drug administration. In that there appeared to be mitoxantrone activity against a salivary gland cancer in one Phase II clinical trial (1), these distribution data provide a rationale for carrying out additional trials of mitoxantrone in the treatment of these relatively rare tumors. Because the concentrations of mitoxantrone in saliva were quite low, it is unlikely that further monitoring of this elimination route would prove useful in the evaluation of mitoxantrone pharmacokinetics.

The mitoxantrone tissue disposition data obtained in 4 of our patients suggest a direct relationship between the degree of drug uptake into tumor or normal tissue cells and biological effect. Although metastatic melanoma cells obtained prior to therapy in one of our patients had proven exquisitely sensitive to mitoxantrone in vitro (14), treatment was associated with progressive disease and only pg quantities of ^{14}C-labeled drug equivalents per $1 \times 10^6$ cells were recovered approximately 23 h after drug administration. Thus, despite evidence of inherent tumor sensitivity to mitoxantrone, clinical response obviously

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[4] D. Woodward (American Cyanamid Co., Pearl River, NY), personal communication.

depends on adequate drug uptake into target tissue. Drug disposition studies of this type can provide important insights when interpreting the results of Phase II clinical trials.

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