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Pharmacological disposition of 1,4-dihydroxy-5-8-bis[[2[(2-hydroxyethal)amino] ethyl]amino]-9,10-anthracenedione dihydrochloride in the dog

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Summary. *DHAQ, a new antitumor agent, has been selected for clinical trial on the basis of its activity against a number of transplantable rodent tumors. In anticipation of the clinical trial of this agent, the pharmacology of DHAQ was studied in beagles by high-pressure liquid chromatographic and radiochemical techniques that are specific for the unchanged drug. ^{14}C-DHAQ was administred IV to beagles at a dose of 5 mg/kg, 100–125 μCi total. With a maximal plasma concentration of 75 ± 2.7 ng/ml, DHAQ was eliminated from the plasma with a half-life of 28.1 h during the terminal phase. The total clearance of DHAQ was 10.1 ± 0.4 mg/kg/min, while the apparent volume of distribution was 26.6 ± 4.9 l/kg. In 48 h, 2.4% ± 0.6% of the dose was excreted in the urine and 3.0% ± 0.1% in the bile as the unchanged drug. At autopsy performed 5 h after dosing, the highest percentage of the administered DHAQ was in the liver (49.7% ± 2.7%), followed by the small intestine (7.1% ± 0.7%), kidneys (2.7% ± 0.1%), lung (1.9% ± 0.3%), spleen (1.6% ± 0.3%), and stomach (1.3% ± 0.1%). The heart, large intestine, pancreas, gallbladder, urinary bladder, and brain each retained less than 1% of the dose. However, 24 h after dosing 10.6% of the drug was detected in the liver and 2.9% in the small intestine. In terms of the percentage of the dose, the distribution of DHAQ in the other organs either remained unchanged or increased slightly. In concentrations varying from 10 ng/ml to 10 μg/ml the drug was 70%–80% bound to plasma protein. DHAQ was metabolized to two unidentified metabolites. Thus, this drug appeared to be cleared from the plasma of beagle dogs chiefly by tissue binding, leading to possible persistence of the drug in certain body compartments.*

Introduction

Attempts to circumvent the myocardial toxicity of the antitumor anthracycline antibiotics culminated in the synthesis of a series of bis(substituted aminoalkylamino)-9,10-anthracenediones bearing some structural resemblance to the anthracyclines. Among these novel anthracenediones, one in particular, DHAQ (NSC-279836, mitoxantrone), has exhibited impressive activity against experimental tumors, including a few cures [5]. These encouraging results prompted clinical trials of DHAQ at several centers. We have therefore studied the pharmacologic disposition of this drug in beagle dogs, with the hope that such studies may aid its current clinical trials. Similar studies with HAQ, a structurally closely realted agent, have already been described [6].

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Material and methods

Drugs and reagents. DHAQ (NSC-279836) and radioactive [2-hydroxyethyl-^{14}C]DHAQ (specific activity 11.2 mCi/mmol, 95% radiochemically pure by radioautography) were supplied by the Drug Development Branch, Divison of Cancer Treatment, National Cancer Institute. Glass-distilled chromatographic solvents were purchased from Burdick Jackson Laboratories, Muskegon, Mich. Other chemicals and reagents were obtained from regular commercial sources.

Radiochemical techniques. Radioactivity was determined with a Packard model 2650 Tri-carb Liquid Scintillation Spectrometer equipped with an automatic self-calibration quenching correcting device and capable of direct computation of disintegrations per minute (dpm). Plasma and urine samples of 0.2 ml each were counted in 11 ml PCS, a commercial phase-combining counting solution available from Amersham Corporation, Arlington Heights, IL. Tissues were combusted in a Packard model B306 sample oxidizer; the $^{14}CO_2$ generated was trapped in 5–10 ml Carbo-sorb and counted after mixing with 11–15 ml Permafluor V, both Packard products.

Chromatography. Analyses were carried out with a Waters Associates model 204 liquid chromatograph in essentially the same manner as previously reported for HAQ [6]. For analysis of very small amounts of radioactive DHAQ, unlabeled drug was added to the samples as a marker. This allowed monitoring of the absorbance of these samples at 254 nm. In such cases, the eluent was collected at 2-min intervals for counting. The recovery of the radioactivity by this procedure was greater than 90%.

Extraction procedure. Generally, the method of extraction followed closely the procedure for the extraction of HAQ from biological specimens [6]. Plasma was deproteinated with one-tenth of its volume of 20% sulfosalicylic acid, centrifuged, and the supernatant was made alkaline to pH 10 with 8 *N*-NaOH. Urine was collected as voided, and similarly made alkaline before extraction. To extract [^{14}C]DHAQ from tissues, a 10-g specimen was blotted dry, minced with scissors, and mixed with 10 ml 1 *N*-NaOH. The mixture was placed in a 50-ml beaker, cooled in an ice bath, and homogenized with a model PT 10 Polytron tissue homogenizer (Brinkmann Instru-

ments) at 22,000 rpm for 10 min. The homogenate was centrifuged at 12,000 *g* for 10 min, and the supernatant deproteinated and extracted as above with plasma; the recovery of [^{14}C]DHAQ was 75%–80%.

Dogs. Beagles of both sexes weighing 8–12 kg, were used in these studies. [^{14}C]DHAQ in sterile water (5 mg/ml) was administered at 5 mg/kg IV over 15 min by the femoral vein while the animal was under pentobarbital anesthesia. The collection and processing of blood, urine, bile, cerebrospinal fluid (CSF), and tissue samples followed the same procedure as that described previously for HAQ [6], except that in the 48-h studies urine was collected as voided from unanesthetized animals in metabolic cages. DHAQ in the specimens was determined as outlined above.

Pharmacokinetic computations. Experimental results were subjected to computerized nonlinear regression analysis. Best-fit criteria were based on correlation coefficient determination and the F-test [2]. Pharmacokinetic parameters were computed by standard techniques [4].

Protein binding. Binding of DHAQ with dog plasma protein was determined by equilibrium dialysis [3] at 25° C for 24 h; DHAQ was stable under these experimental conditions.

Results

Pharmacokinetics

The methodology used in the present study was sensitive enough to permit the analysis of plasma for DHAQ in concentrations as low as 5 ng/ml, thus enabling us to carry out 48-h experiments. After IV administration of [^{14}C]DHAQ at 5 mg/kg (100–125 μCi total) the unchanged drug was cleared from the plasma triphasically (Table 1 and Fig. 1), with an average initial half-life of 6.5 min, a middle half-life of 1.3 h, and a terminal half-life of 28.1 h; the highest plasma drug concentration was 28.3 μ*M* (12.6 μg/ml). The mean apparent

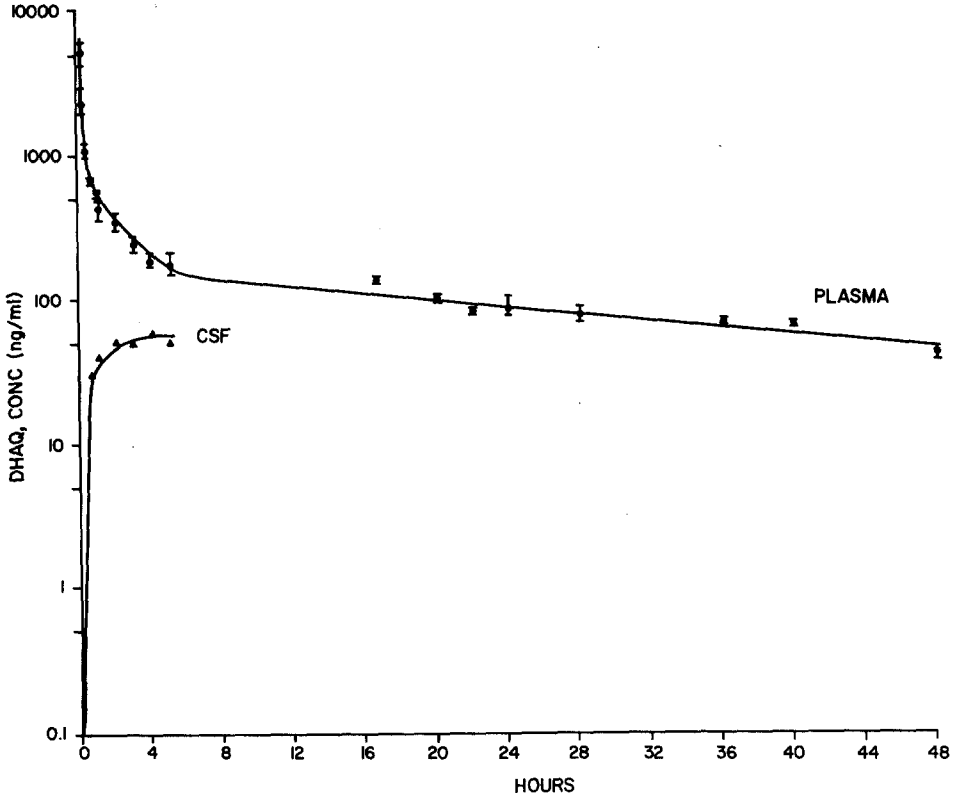

Fig. 1. Average plasma and CSF DHAQ concentrations in beagles after IV administration of 5 mg/kg

Table 1. Pharmacokinetic parameters of DHAQ

Dog no.	Plasma peak conc. (μg/ml)	Plasma half-lives			Volume of distribution[b] (1/kg)	Total clearance (ml/kg/min)	Excretion in 48 h	
		α (min)	β (h)	γ (h)			Urine	Bile
							% of dose	
1	4.6	5.5	1.3	28.6	26.5	10.7	3.6	3.2
2	12.6	6.4	1.6	20.5	18.1	10.2	2.1	2.8
3	5.1	8.0	1.0	43.6	35.2	9.3	1.6	3.0
Mean ± SE	7.5 ± 2.6	6.5 ± 0.7[a]	1.3 ± 0.1[a]	28.1 ± 7.1[a]	26.6 ± 4.9	10.1 ± 0.4	2.4 ± 0.6	3.0 ± 0.1

[a] Harmonic mean
[b] Volume of distribution by the area method

volume of distribution was 26.6 l/kg. The total clearance of DHAQ was 10.1 ml/kg/min, as compared with clearance of 4 ml/kg/min for creatinine in this species (Table 1).

In 48 h, 4.9% of the administered radioactivity was recovered in the urine, including 2.4% as the unchanged drug (Fig. 2). Additionally, 4% of the administered radioactivity was excreted in the bile, including 3% as DHAQ (Fig. 2). DHAQ penetrated the CNS to some extent (Fig. 1); unfortunately, after 5 h, no further satisfactory CSF specimens were obtainable by cisternal puncture.

In concentrations of 10 ng/ml to 10 µg/ml, DHAQ was 70%–80% bound to dog plasma protein.

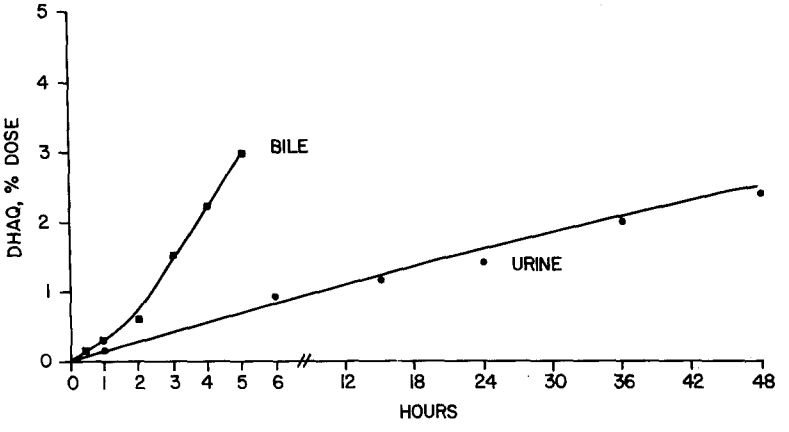

Fig. 2. Average cumulative excretion of DHAQ in urine and bile after IV administration of 5 mg/kg

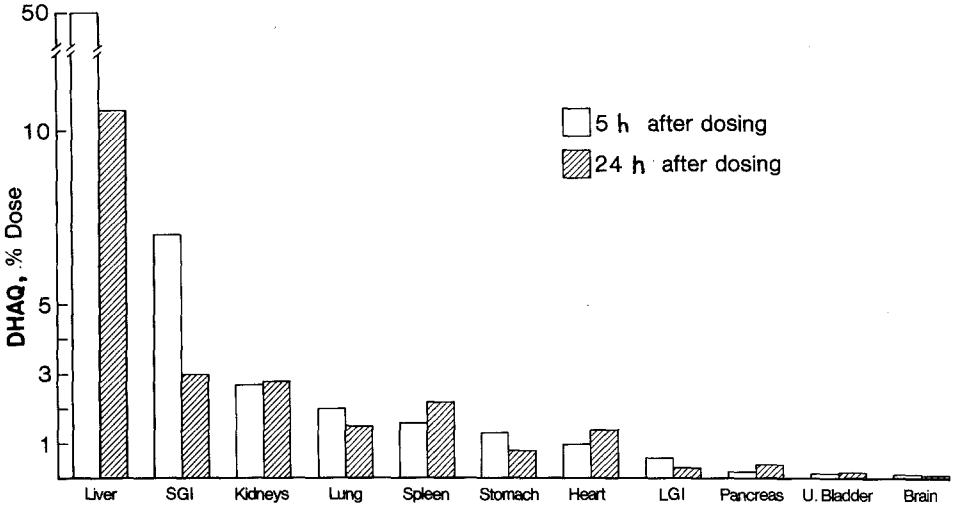

Fig. 3. Average distribution of percentage of dose of DHAQ in organs after IV administration of 5 mg/kg

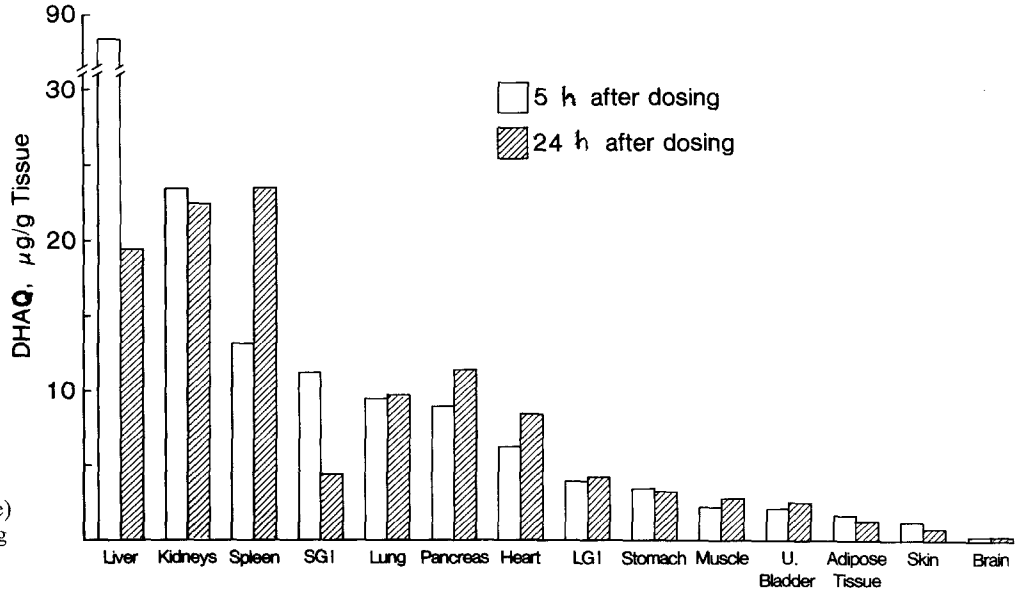

Fig. 4. Average distribution of DHAQ in organs (µg/g wet tissue) after IV administration of 5 mg/kg

Distribution

At autopsy performed 5 h after dosing, the liver had accumulated the highest percentage of the administered dose as the unchanged drug (49.7% ± 2.7%) (Fig. 3), followed by the small intestine, kidneys, lung, spleen, and stomach. The heart, large intestine, pancreas, gallbladder, urinary bladder, and brain each retained less than 1% of the dose. However, 24 h after dosing, only 10.6% of the drug was detected in the liver and 2.9% in the small intestine. In the other organs the percentage of the dose of DHAQ either remained unchanged or appeared to have increased. Expressed in μg/g wet tissue, that DHAQ distribution assumed a different pattern. Figure 4 shows that 5 h after drug administration the liver, kidneys, spleen, small intestine, lung, and pancreas exhibited DHAQ concentrations of 10 ng/ml or more. Drug concentrations in the heart, large intestine, stomach, muscle, urinary bladder, adipose tissue, and skin were between 1 and 7 ng/ml. After 24 h, concentrations of DHAQ in the liver and small intestine diminished substantially; however, in the other organs the concentrations of the drug either remained virtually the same or apparently increased.

Metabolism

DHAQ was biotransformed into two metabolites that have not yet been identified. In the plasma, an average of 65% (57%–77%) of the radioactivity resided in unchanged DHAQ, while in the urine approximately 50% of the radioactivity was in unchanged drug.

Discussion

Overall, the pharmacokinetics of DHAQ were similar in man [7] and dogs. In both species the plasma half-life of the drug was relatively long. The total clearance of DHAQ was at least twice that of creatinine in man and dogs, but the contribution to this parameter by the renal route was minor since the urinary excretion of DHAQ was very low. Among the several possible explanations for this apparent paradox, localization of the drug in certain tissues is probably the most likely. First of all, the large apparent volume of distribution found with DHAQ is compatible with drug localization in tissues. Secondly, in these experiments a large percentage of DHAQ was bound to protein in the plasma, suggesting that binding to tissues was also high. Furthermore, the studies on drug distribution provide further support for this contention. At 24 h after drug administration, at least 25% of the dose remained in various organs. Lastly, we recently found that DHAQ was preferentially taken by nucleated white blood cells, particularly the polymorphonuclear neutrophils (N. Savaraj et al., in preparation).

In addition, metabolic clearance and biliary excretion must have contributed considerably to the total clearance of the drug. We previously determined that HAQ, a close analog of DHAQ differing from it only in the absence of the 1,4-dihydroxy groups, was excreted in the bile of the dog to the extent of almost 40% of the dose in 5 h [6]. In the present study, unfortunately, it was impractical for us to collect the bile from the animal beyond 5 h, during which time merely 3% of the dose was found in the bile. However, from Fig. 2 it appears highly likely that by 24 h considerably more DHAQ would have been excreted in the bile. In any event, since very little of the administered DHAQ found its way in the urine, changes in renal function probably would not significantly affect the pharmacokinetics and toxicity of this agent. In this regard, DHAQ somewhat resembles doxorubicin [1], although the structural similarities between these two compounds are minimal.

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