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Pharmacokinetics of homoharringtonine in dogs

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Summary. We studied the pharmacokinetics and distribution of homoharringtonine (HHT), an antitumor alkaloid, in anesthetized dogs using chromatographic and radiochemical techniques. Uniformly tritiated HHT was administered i.v. to five dogs at doses of 0.05 to 0.34 mg/kg, 200 μCi per animal. Unchanged HHT disappeared in a triphasic manner from the plasma with an initial plasma $t_{1/2}$ of 9.4±4.2 min, an intermediary $t_{1/2}$ of 1.4±0.5 h, and a terminal $t_{1/2}$ of 40.6±4.6 h. The plasma clearance was 114.0±20.1 ml/kg^{-1} h^{-1} and the steady-state volume of distribution was 6.2±0.7 l/kg. In 72 h, 40.1%±4.0% of the administered radioactivity was excreted in the urine, 17.8%±2.7% of which was unchanged HHT. HHT was metabolized extensively to one major and two minor metabolites. Biliary excretion of total radioactivity was 14.4% in 5 h, 2% of which was HHT. HHT concentration in the CSF was highest 4 h after drug administration, about 40% of the concentration in the concurrent plasma. At autopsy 5 h after dosing, the highest percentage of HHT was in the liver (7.4%), followed by the small intestine (2.5%), stomach (1.0%), pancreas (0.8%), kidneys (0.8%), and lungs (0.7%). The heart, spleen, large intestine, and brain each retained less than 0.5%. However, 24 h after dosing, 4% of the HHT still remained in the liver, 1% in the small intestine, and less than 1% in the other organs. HHT seems to be extensively metabolized in dogs and partially retained in the body.

Introduction

Homoharringtonine (HHT, NSC-141633) is one of a group of cephalotaxine esters that have shown antitumor activity against murine P388 lymphocytic leukemia. The cephalotoxines were originally isolated by Powell and co-workers from wood, bark, and twig samples of *Cephalotaxus harringtonia*, an evergreen plant native to mainland China [5, 6]. In 1976, the Chinese reported that two preparations of cephalotoxine esters containing different proportions of HHT and harringtonine had been isolated from *C. fartunei* and had induced complete and partial remissions in patients with acute leukemia [1]. This result has been partially confirmed in the United States [4, 9]. Both HHT and harringtonine demonstrated high activity against colon 38 tumor and P388 leukemia, moderate activity against the CD8F mammary carcinoma and L1210 leukemia, and only slight activity against B16 melanocarcinoma. Possibly, both HHT and harringtonine exert their antitumor activity by inhibition of protein synthesis [2, 8]. HHT was selected for phase 1 trial by NCI not only because high yields could be obtained from plant materials, but also because it showed significant preclinical antitumor activity. Toxic effects in the phase 1 trial included myelosuppression, hypotension, and tachycardia; the maximum tolerated dose was 4 mg/m^2 for 5 days [3]. However, little is known about the pharmacokinetics of this drug. Only one pharmacokinetic study has appeared to date in the Chinese literature, and the methodology used and results presented are not at all clear. Therefore, we chose to study the pharmacokinetics of HHT concurrently in patients and dogs; the clinical study has already been published [7]. We now report on our pharmacokinetic studies in dogs; some of the findings were at variance with those found in man.

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Materials and methods

Drugs

Uniformly labeled [^{3}H]HHT was prepared by Amersham Corporation (Arlington Hts., Ill, USA) with a radiochemical purity of 85% as assessed by HPLC. The specific activity of this preparation was 191 mCi/mmol. This material was free of exchangeable tritium. Unlabeled homoharringtonine was supplied by the Division of Cancer Treatment, NCI. Glass-distilled chromatographic solvents were purchased from Burdick Jackson Laboratories (Muskegon, Mich, USA). Other chemicals and reagents were of analytical grade and were obtained from regular commercial sources.

Dogs

Mongrel dogs of either sex, average weight 20 kg, were lightly anesthetized with pentobarbital. They received 0.05–0.35 mg/kg (200 μCi total) HHT through the femoral vein in 10 min. Blood samples, 10 ml each, were collected at predetermined intervals into tubes containing sodium heparin as the anticoagulant. They were immediately centrifuged at 12,000 *g* for 10 min at 25° C. The plasma was separated from the cells and frozen until analysis. CSF samples were collected by cisternal puncture, and urine was collected through a Foley catheter. Bile was sampled by cannulation of the common bile duct. At the end of the

experiment, the dogs were killed with an overdose of pentobarbital. At autopsy, representative tissue specimens of 1–2 g were removed from various vital organs for HHT assay.

Radiochemical technique

Total radioactivity was determined with a Packard model 2650 Tri-Carb Liquid scintillation spectrometer equipped with an automatic self-calibration, quench correction device, which was capable of directly determining rates of disintegration per min (dpm). Total radioactivity represents combined radioactivities of both HHT and metabolites.

Samples of biological fluid, 0.2 ml each, were counted in 11 ml PCS, a commercial phase-combining counting solution available from Amersham Corporation (Arlington Hts., Ill, USA) Tissues (0.2–0.5 g) were combusted in a Packard model 306B Sample Oxidizer; the tritiated water generated was trapped in Monophase, also a Packard product, and counted in the Tri-Carb Liquid scintillation spectrometer.

Determination of unchanged HHT

Extract procedure. Samples of plasma, urine, or bile, 1 ml each, were extracted twice with 6 ml methylene chloride and centrifuged at 12,000 *g*. The combined organic phases were evaporated to dryness in a stream of nitrogen. The residue was reconstituted with 500 µl mobile phase, and 350 µl was applied to HPLC for determination of the parent drug. The recovery of the radioactivity was better than 85%.

To extract HHT from tissues, a 1- to 2-g specimen was rinsed with 0.9% NaCl, blotted dry, minced with scissors, and mixed with 10 ml 0.9% NaCl. The mixture was placed in a flask, cooled with ice, and homogenized with a model PT-10 Polytron Tissue Homogenizer (Brinkman Instruments, NY, USA) at 27,000 rpm for 10 min. The homogenate was extracted three times with methylene chloride and the combined organic phases were dried, reconstituted with mobile phase, and centrifuged in a microfuge (Fisher 235) for 5 min. The supernatant was subjected to HPLC analysis for HHT. The recovery of [^{3}H]HHT from tissue was 80%±5%, compared with the total radioactivity of the oxidized sample.

Chromatography. Analyses were carried out on a Waters Associates model 204 high-pressure liquid chromatograph (Milford, Mass, USA) using a µBondapak C_{18} reverse-phase column (30 cm × 4.0 mm). Samples were eluted isocratically, using 0.1 *M* ammonium formate (pH 6.0) in 40% MeOH. The flow rate was 2.0 ml/min and the UV monitor was set at 284 nm at a sensitivity setting of 0.02 AUFS [6].

Sufficient unlabeled HHT was added to all samples as a marker, and the eluent was collected at 1 min intervals. Whenever the presence of HHT was detected by UV absorbance peak at 284 nm, the entire volume of that peak was collected, mixed with PCS, and counted in the scintillation counter. In this system, HHT was eluted at 12 min, whereas a major radioactive metabolite was eluted at 6 min. Moreover, two minor extractable metabolites were eluted at 30 and 38 min.

HHT standard curve. An appropriate stock solution of HHT was prepared; 0.1-ml portions of the stock solution were used in the preparation of serial dilutions with individual dog plasmas. Samples were extracted as above and analyzed for HHT concentration as described above. The standard curve was linear, from 5 to 100 ng/ml, with a lowest limit of detection of 5 ng/ml.

Pharmacokinetic analyses. The plasma HHT data were subjected to nonlinear regression analysis with the aid of the CYBER 174 computer system, using the ESTRIP and NONLIN programs. The data points were appropriately weighted with reciprocal drug concentrations. Best-fit criteria were determined by the *F*-test; the three-compartment open model gave the best results.

Results

Pharmacokinetics

After i.v. administration of [^{3}H]HHT, the unchanged drug was cleared from the dog's plasma triphasically. The pharmacokinetic parameters of unchanged HHT are shown in Table 1. Figure 1 shows the results of a typical experiment; an average initial $t_{1/2}$ of 9.4±1.2 min, an intermediate $t_{1/2}$ of 1.4±0.5 h, and a terminal $t_{1/2}$ of 40.6±4.6 h were observed. The apparent mean, steady-state volume of distribution was 6.2±0.7 l/kg, and the total clearance was 114.0±20.1 ml·kg^{-1} h^{-1}. In 72 h, 40.1%±4.0% of the administered radioactivity was recovered in the urine, of which 17.8%±2.7% was unchanged drug. Biliary excretion of tritium was 14.4% in 5 h, with 2% as HHT (Fig. 2). HHT concentration in the CSF was highest 4 h after drug administration and was about 40% of that in the concurrent plasma sample (Fig. 1). As in our studies in patients, a major radioactive metabolite was detected in the plasma, urine, and bile in all of the experiments. Additionally, two labeled metabolites of lesser quantity were also found occasionally in urine and bile.

Table 1. Pharmacokinetic parameters of unchanged HHT

Dose	$t_{1/2}$			Cl	Vd	Urinary excretion
mg/kg^{-1}	min	h	h	ml/kg^{-1} h^{-1}	l kg^{-1}	% dose in 72 h
0.05	4.4	0.4	32.2	112.2	5.2	21.7
0.25	12.5	1.3	37.1	152.3	8.1	8.3
0.25	1.5	1.1	31.9	164.7	7.6	15.6
0.25	4.0	0.7	46.0	80.4	5.3	23.0
0.35	24.7	3.4	55.9	60.4	4.9	20.5
Mean ± SE	9.4±4.2	1.4±0.5	40.6±4.6	114.0±20.1	6.2±0.7	17.8±2.7

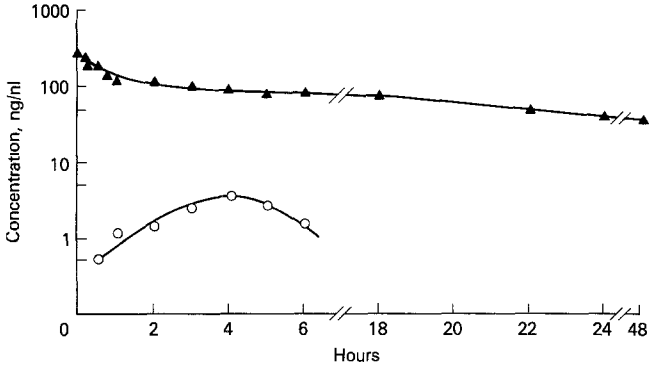

Fig. 1. Plasma clearance of HHT in a typical dog. The animal received 0.35 mg/kg HHT i.v. The experimental data points were: ▲, Plasma; ○, CSF; the curve was computer-generated

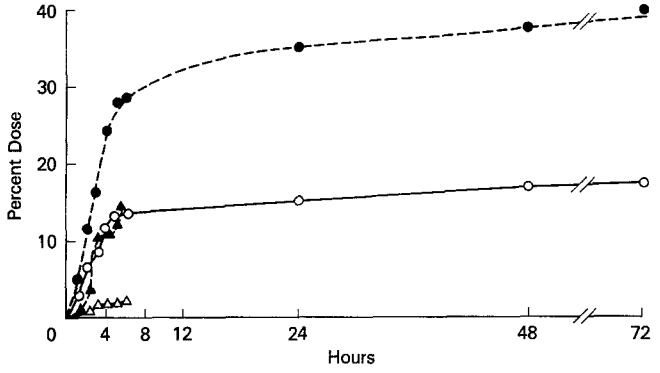

Fig. 2. Average cumulative urinary and biliary excretion of [^{3}H] and HHT in eight dogs. ●, [^{3}H] in urine; ▲, [^{3}H] in bile; ○, unchanged HHT in urine; △, unchanged HHT in bile

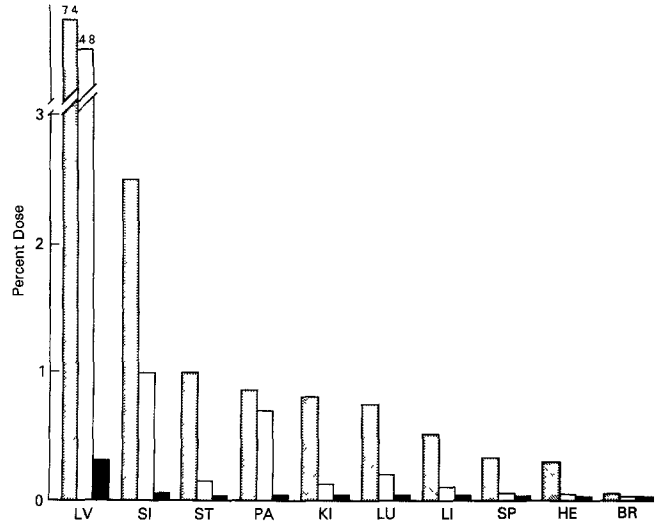

Fig. 3. Accumulation and distribution of HHT in body organs. Dogs were given 0.35 mg/kg HHT by i.v. route. *LV*, liver; *SI*, small intestine; *ST*, stomach; *PA*, pancreas; *KI*, kidney; *LU*, lung; *LI*, large intestine; *SP*, spleen; *HE*, heart; *BR*, brain; □, 5 h; □, 24 h; ■, 72 h

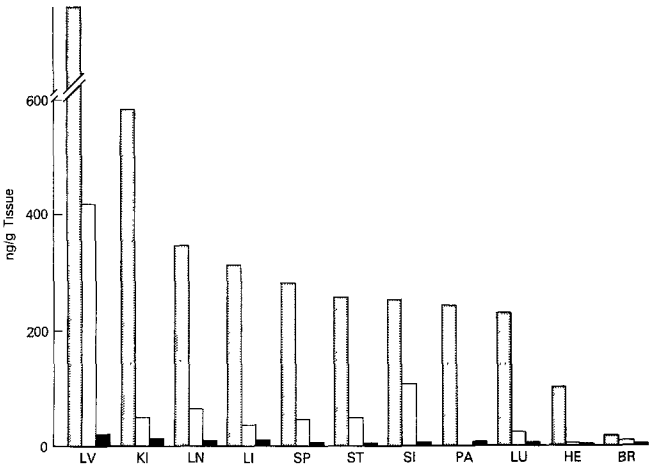

Fig. 4. Tissue levels of HHT after dogs received 0.35 mg/kg i.v. *LV*, liver; *KI*, kidney; *LN*, lymph nodes; *LI*, large intestine; *PA*, pancreas; *ST*, stomach; *SI*, small intestine; *PA*, pancreas; *LU*, lung; *HE*, heart; *BR*, brain; □, 5 h; □, 24 h; ■, 72 h

Tissue distribution

Autopsy was performed 5 h after dosing in three dogs. The liver had accumulated the highest percentage of the unchanged drug (7.4%). The next highest percentage was found in the small intestine (2.5%), followed by the stomach (1.0%), pancreas (0.8%), kidneys (0.8%), and lungs (0.7%) (Fig. 3). The heart, spleen, large intestine, and brain each retained less than 0.5% of the original dose. Twenty-four hours after dosing, 4.8% of the HHT still remained in the liver, 1% in the small intestine, and less than 1% in other organs; however, at 72 h, only 0.3% remained in the liver and less than 0.1% in other organs. When expressed in ng/g west tissue, the distribution of HHT assumed a slightly different pattern. Five hours after dosing, the liver, kidney, lymph nodes, large intestine, spleen, stomach, small intestine, pancreas, and lung retained more than 200 ng/g HHT, and the heart and brain had less than 100 ng/g HHT (Fig. 4). After 24 h, except in the liver, all the organs had less than 100 ng/g HHT. At 72 h, essentially all HHT had vanished from the tissues.

Excretion

The average 72-h, cumulative urinary excretion of HHT was 17.8%±2.7% of the dose, yet that of the total tritium was 39.9%±4.7%. The metabolites were therefore responsible for over 55% of the dose excreted in the urine. Biliary excretion is not a major route of excretion for the parent drug; also, about 12% of the dose was found to be metabolites.

Discussion

HHT is clearly an active anticancer agent, especially for the treatment of acute leukemia [1, 4, 9]; however, little reliable information is available on its disposition and metabolism. We have therefore undertaken concurrent pharmacokinetic studies of this interesting agent in man and dogs; the results of our clinical investigations have already been published [7]. In the present study in dogs, we have shown that during the terminal phase, the plasma half-life of HHT was relatively long compared with that in man,

namely, 40.6 h versus 9.3 h [7]. The apparent steady-state volume of distribution was also much larger in dogs than in man, 6.2 versus 1.8 l/kg; however, the total clearance was comparable in both species, that is, 114 ml/kg^{-1} h^{-1} in dogs versus 177.4 ml/kg^{-1} h^{-1} in man. Thus, HHT tended to localize in some body compartment, perhaps to a somewhat larger extent in dogs than in man. Since the contribution of renal clearance to the total clearance of HHT in the dog was minor (approximately 7%) and that of biliary clearance was even much less (about 0.3%), evidently metabolism and tissue sequestration must be the principal route of HHT clearance. This contention is borne out by the results of our studies. First, the large apparent volume of distribution suggests tissue localization of HHT. Second, 5 h after i.v. administration of HHT at 0.35 mg/kg, at least 14% of the dose was distributed in the major organs (Fig. 3). At that time, the specific amount of unchanged HHT in nanograms per gram of tissue were 680 in the liver and 570 in the kidney. The dose administered, however, was only 0.35 mg/kg or 350 ng/g. This means, at least in these two organs, that HHT was concentrated and retained for a minimum of 5 h. Third, 5 h after drug administration, the plasma concentration of HHT was approximately 90 ng/ml (Fig. 1). But in all the major organs that we studied (Fig. 4) except the brain and possibly the heart, HHT concentrations were significantly higher, assuming that the water content was 100% in all organs. This means that these organs were capable of extracting HHT from the plasma. Undoubtedly, tissue extraction contributed considerably to the total plasma clearance of HHT. Finally, from Figs. 2 and 3 it is readily apparent that HHT was rapidly and extensively metabolized in the dog. As much as 55% of the radioactivity in the urine, and a considerably higher percentage in the bile, was attributable to the metabolites. Thus, our evidence indicates that HHT was cleared from the dog chiefly by tissue binding and metabolism.

In phase 1 clinical trials, hypotension and tachycardia elicited by HHT were the acute limiting toxicities [3]. Surprisingly, we did not observe this phenomenon in dogs when HHT was given as an i.v. bolus. Hypotension that was not life-threatening may have happened later than 6 h after dosing or after repeated dosing. Unfortunately, we could not monitor the blood pressure in dogs every hour for 24 h after HHT administration.

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