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METABOLISM AND PHARMACOKINETICS OF N^1,N^{14}-DIETHYLHOMOSPERMINE

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ABSTRACT:

The pharmacokinetics and metabolism of N^1,N^{14}-diethylhomospermine (DEHSPM) is described. Analysis of 15 min constant rate intravenous infusion data in dogs gave mean values of: plasma $t_{1/2}$ = 1.04 hr; V_d = 0.514 liter/kg; CL = 0.343 liter/hr/kg; and $AUC_{0-\infty}$ = 43.2 mg/hr/liter. The renal $t_{1/2}$ = 0.99 hr, with 36% of the drug recovered in the urine between 0–4 hr unchanged. In other experiments, the drug was administered to dogs by subcutaneous injection. Noncompartmental analysis of plasma concentration-time data showed a mean residence time (MRT) of 4.67 hr (subcutaneous) *vs.* 1.93 hr (intravenous). Mice and dogs received DEHSPM chronically to evaluate tissue distribution of DEHSPM and its metabolites. All tissues examined contained DEHSPM and its *N*-deethylated metabolites, N^1-ethylhomospermine (MEHSPM) and homospermine (HSPM). On day 1 posttreatment, 35% of the total dose administered to mice was present in the liver (25%) and kidney (10%). The DEHSPM present declines rapidly (liver $t_{1/2}$ = 1.6 days). The majority of the original dose was present as HSPM, which persisted in tissues for weeks (liver $t_{1/2}$ = 15.4 days). These data suggest that DEHSPM and MEHSPM are metabolized by *N*-deethylation, but that HSPM is not susceptible to further degradation by polyamine catabolic enzymes that involves stepwise removal of aminopropyl equivalents by spermine/spermidine N^1-acetyltransferase/polyamine oxidase. Thus, chronic DEHSPM dosing regimens in both dogs and mice may result in the accumulation of HSPM, which is retained by tissues for an extended period of time resulting in disruption of normal polyamine homeostasis in these tissues. These findings correlate with clinical and histopathological signs of toxicity in dogs and in mice.

Our laboratory has developed a series of *N*-terminally dialkylated analogs and homologs of the naturally occurring tetraamine, SPM.[1] These compounds exhibit antineoplastic activity against a number of murine and human tumor lines both *in vitro* and *in vivo* (1–3). Interestingly, very small structural alterations in these polyamine analogs can result in marked differences in their biological activities (4, 5). One of these, DENSPM, is currently in phase I clinical trials as an antineoplastic. A second member of this series, DEHSPM, was found to be a potent inhibitor of gastrointestinal motility during early clinical evaluation as an antineoplastic. Subsequent electrophysiological studies in rats and isolated guinea pig ileum strips showed that DEHSPM was able to abolish the waves of myoelectric activity associated with peristalsis (6). These observations have led to an ongoing clinical evaluation of DEHSPM in the control of acquired immune deficiency syndrome-related chronic diarrhea.

We have recently reported the pharmacokinetics and metabolic disposition of DENSPM (7). To understand further the basis for the differences in biological properties between DEHSPM and DENSPM and to support the ongoing clinical studies, we now report the pharmacokinetics and metabolic disposition of DEHSPM in the dog and mouse.

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[1] Abbreviations used are: SPM, spermine; DENSPM, N^1,N^{11}-diethylnorspermine; DEHSPM, N^1,N^{14}-diethylhomospermine; PUT, putrescine; SPD, spermidine; MEHSPM, N^1-ethylhomospermine; HSPM, homospermine; tid, three doses/day; BUN, blood urea nitrogen; k_{el}, terminal elimination rate constant; $t_{1/2}$, terminal elimination half-life; AUC, area under the concentration-time curve; V_d, volume of distribution; CL, clearance; MRT, mean residence time; NSPM, norspermine; LD_{50}, median lethal dose; AdoMetDC, *S*-adenosylmethionine decarboxylase; SSAT, spermine/spermidine N^1-acetyltransferase; PAO, polyamine oxidase.

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Materials and Methods

L-Proline, PUT, SPD, SPM, 1,6-diaminohexane, and 1,7-diaminoheptane were obtained from Aldrich Chemical Company (Milwaukee, WI). Reagent-grade hydrochloric acid, perchloric acid, and anhydrous sodium carbonate were obtained from Fisher Scientific (Pittsburgh, PA). All other solvents were Fisher Optima grade. Dansyl chloride (99%) was purchased from Lancaster Synthesis, Inc. (Windham, NH). DEHSPM, MEHSPM, and HSPM were synthesized *via* a previously published route (1). All animals were obtained from Harlan Sprague-Dawley (Indianapolis, IN). The rodent studies were conducted using CD-1 female mice of 10–12 weeks of age selected at random.

Acute and Chronic Toxicity of DEHSPM and Its Metabolites in Mice. For acute toxicities, the polyamine analogs were administered as a single intravenous injection to groups of six animals at each dose. The animals were scored as "alive" or "dead" 2 hr after administration of drug. All animals were observed for a period of 10 days after the treatment.

In the chronic toxicity regimen, mice were administered the polyamine analog as tid for 6 days for a total of 18 injections/animal. Appetite, weight, and overall physical appearance were recorded at the time of each injection. After the treatment regimen had been completed, animals were observed an additional 10 days. The chronic toxicity dosing regimen, based on the acute toxicity results, was set up so that at least one test group presented with a high fraction of lethalities.

Tissue Distribution of DEHSPM and Metabolites in Mice. To assess the metabolic fate of DEHSPM and potential relationships to chronic toxicity, two studies were conducted in mice. One study examined the accumulation of DEHSPM and its metabolites during a chronic treatment regimen. The second study examined the distribution and rate of disappearance of DEHSPM and metabolites after cessation of a chronic treatment regimen.

DEHSPM (6 mg/ml) was dissolved in sterile water for injection and filtered through a 0.2 μm filter (Whatman) before use. Control mice received no

DEHSPM. Various tissues, including the liver and kidney, were prepared for HPLC analysis of polyamine content. To facilitate their handling, the organs were submerged in liquid nitrogen. Once frozen and weighed, the tissues were homogenized (Tissuemizer, Tekmar, Cincinnati, OH) in 1.2 N perchloric acid (containing 1,7-diaminoheptane internal standard) in a 1:20 (w/v) ratio. The tissue homogenates were then freeze-thawed 3 times and stored in a −70°C freezer until HPLC analysis was performed.

The rate of accumulation (buildup) of DEHSPM and metabolites was evaluated in mice administered DEHSPM at a dose of 10 mg/kg ip once a day for 7 days. Animals were killed in groups of three 6 hr after the final dose on days 2, 4, and 7 during the period of treatment.

The rate of disappearance (washout) of DEHSPM and metabolites was evaluated in mice that had been administered DEHSPM at a dose of 15 mg/kg ip once a day for 6 days. This washout experiment was set up to provide a direct comparison to a similar experiment we have previously reported for the related drug, DENSPM (7). Animals were killed in groups of 3 at 1, 3, 8, 10, 14, 21, and 35 days after the final dose.

Tissue Distribution and Toxicity of DEHSPM and Metabolites in Dogs. A protocol was designed to treat dogs chronically (29 days) with DEHSPM. Among the important goals of this study were: *a*) to elicit and carefully document signs of toxicity; and *b*) to examine the tissue distribution of DEHSPM and metabolites, as well as assess their persistence in dog tissues after cessation of treatment. Six male beagles ~4 months old and weighing 6–8 kg were obtained from Harlan Sprague-Dawley. The dogs were divided into three groups of two and injected with an isotonic solution of DEHSPM (pH adjusted to 7.2) subcutaneously twice a day at 12-hr intervals at a dosage of 1, 2, or 4 mg/kg/day, respectively. The animals in the 1 and 2 mg/kg/day groups were given the drug for 29 days, whereas the animals in the 4 mg/kg/day group received the drug for only 21 days, because of the appearance of signs of toxicity. The animals were provided food and water *ad libitum*, were weighed once daily in the mornings, and carefully monitored for signs of toxicity. For the first 2 weeks of the study, all of the animals were gaining weight at a fairly consistent rate. After this time, however, the animals in the 4 mg/kg/day group became anorexic and began to lose weight. The animals in the two lower dosage groups gained weight throughout the study (range: +13% to +21% on day 29).

Blood samples were obtained for routine screening analysis by an outside clinical laboratory (Allied Clinical Laboratories, Gainesville, FL) before initiation of the study and twice a week during and after the treatment period. The blood analysis consisted of a routine cell blood count and a "CHEM 22" panel that included the following: glucose, sodium, potassium, chloride, carbon dioxide, BUN, creatinine, calcium, phosphorus, total protein, albumin, alkaline phosphatase, SGOT, SGPT, total cholesterol, total bilirubin, BUN/creatinine ratio, globulin, A/G ratio, γ-glutamyltransferase, ion gap, and ionized calcium determinations. In addition, a urine sample was obtained *via* catheter once a week throughout the study for microscopic and chemical analyses.

For tissue distribution studies, one animal at each dose level was killed 3 days after the final dose, and the other 10 days after the final treatment. In addition to a standard pathology work-up at necropsy, samples of various tissues, including the liver, kidney, spleen, and lung, were removed and prepared for HPLC analysis as described for mouse tissues.

Dosing and Collecting of Pharmacokinetic Samples in Dogs. Male beagles were maintained on a diet of Purina Lab Chow and water provided *ad libitum*. The dogs were fasted for ~16 hr before a pharmacokinetics experiment and a fasting weight obtained. The dogs were restrained in a sling and had their legs shaved to facilitate blood sampling. A urinary catheter was inserted to allow for urine withdrawal.

Two methods of drug administration were used: intravenous constant rate infusion and subcutaneous injection. For constant rate infusion of the drug, a 23-gauge butterfly catheter was inserted into a cephalic vein and taped into place. The drug solution was infused in saline, not pH-adjusted, at a constant rate using a Harvard Apparatus compact syringe pump. Blood samples were taken pre-, during, and postdrug infusion. A leg vein was used for withdrawal of blood samples for analysis.

The blood samples in 1.8-ml vacutainer tubes containing buffered sodium citrate were centrifuged immediately, and the separated plasma was stored on dry ice. At the conclusion of the experiment, the samples were stored at −20°C until HPLC analysis could be performed. Blood samples were acquired at the following times: 15-min constant rate intravenous infusion—0, 2*, 4*, 6*, 8*, 10*, 15, 20, 25, 30, 35, 40, 50, 60, 75, 90, 105, 120, 150, 180, 210, and 240 min; at 5, 6, and 24 hr; and at 2-hr constant rate intravenous infusion—0, 10*, 20*, 30*, 40*, 50*, 60*, 75*, 90*, 105*, 120, 135, 150, 165, 180, 195, 210, 225, and 240 min; and at 5, 6, and 24 hr. (* These samples were taken during the infusion of the drug.) A dose of 14.8 mg/kg was administered subcutaneously in a shaved area of the hindleg. In the first experiment, plasma samples were taken at 0, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 75, 90, 105, 120, 150, 180, 210, and 240 min, and at 5, 6, 8, 12, and 24 hr. In the second experiment, plasma samples were taken at 0, 15, 30, 60, 90, 120, 150, 180, 210, and 240 min, and at 5, 6, 7, 8, 10, 12, and 24 hr.

Urine samples were collected during the first 4 hr of each pharmacokinetic experiment *via* bladder catheterization using a no. 5 French, 16-inch urethral catheter lubricated with KY-Jelly. For each time point, the urine was aspirated from the bladder. The bladder was then rinsed 2 times with 5 ml of sterile normal saline, and the urine/saline mixture was aspirated. Urine and saline washes were mixed together and their volume determined. After collection, the urine mix was acidified with 1.0 N hydrochloric acid and stored on ice. At the conclusion of the experiment, the samples were stored at −20°C until HPLC

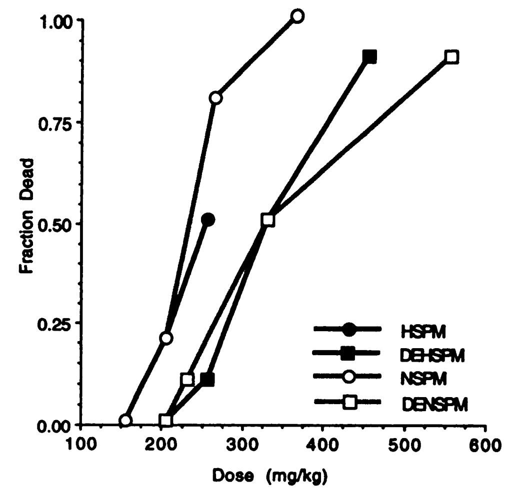

FIG. 1. *Acute toxicities of DEHSPM/DENSPM (N = 10 at each dose) and HSPM/NSPM (N = 5) in mice.*

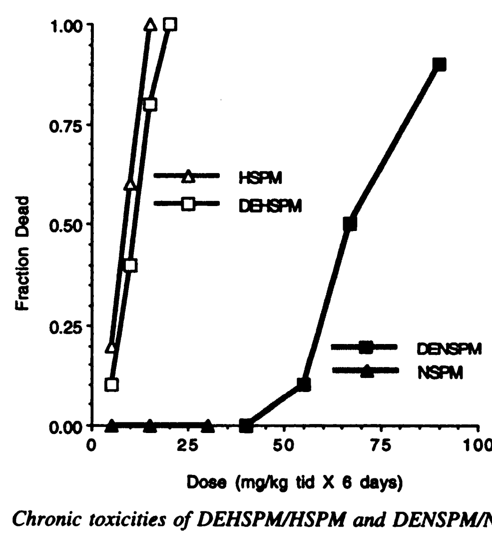

FIG. 2. *Chronic toxicities of DEHSPM/HSPM and DENSPM/NSPM in mice.*

Groups of five female CD-1 mice receive three intraperitoneal injections of drug daily for 6 days (18 doses total). Physical condition of survivors is evaluated daily for 10 days after the final treatment. The fraction dead is calculated by dividing the total number of deaths during the 16-day experiment by the number in the dosage group.

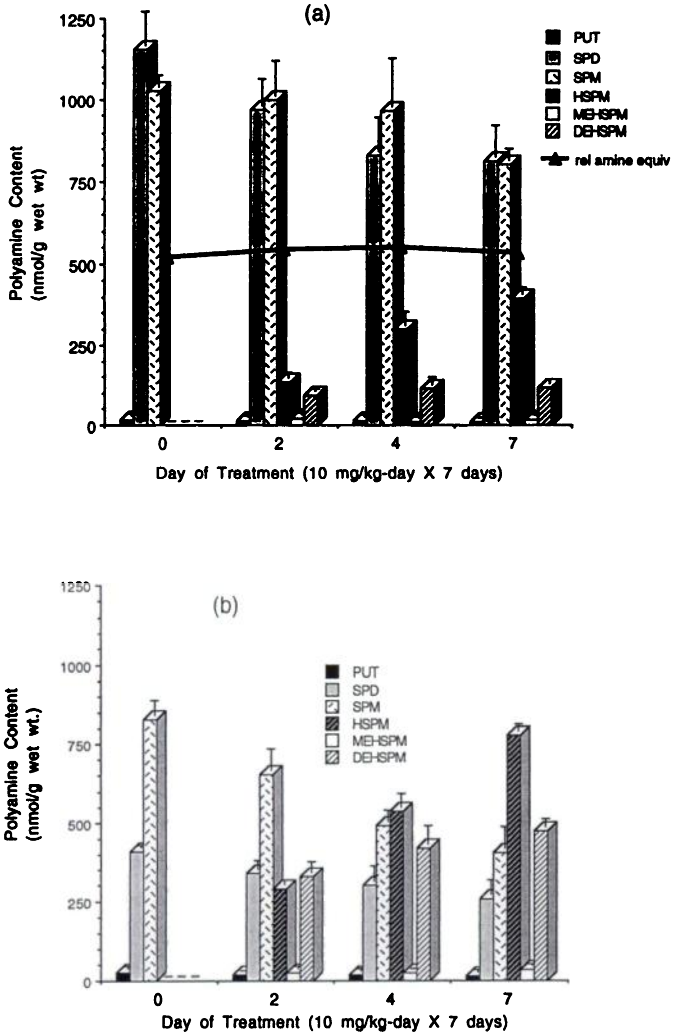

FIG. 3. *Accumulation of DEHSPM and metabolites in mouse liver and kidney during a chronic dosage regimen (10 mg/kg/day DEHSPM as a single intraperitoneal injection daily for 7 days).*

Groups (N = 3; mean ± SD) were killed for HPLC analysis on days 0 (control), 2, 5, and 7 (8 hr after last dose of drug on respective days). Tissue polyamines were determined by HPLC analysis of liver (*a*) and kidney (*b*). Total polyamine N^+ equivalents (nequivalents × 10^{-1}/g wet wt.) are plotted for the liver (*a*). rel amine equiv, relative amine equivalents.

analysis. Urine samples were taken before the drug was administered and at ½-hr intervals for up to 4-hr postdrug.

HPLC Analysis of Polyamines in Tissue, Plasma, and Urine. Each chromatographic assay included calibration standards that were treated in the same manner as the samples. The calibration standards were prepared by adding known amounts of PUT, 1,7-diaminoheptane, SPD, SPM, HSPM, MEHSPM, and DEHSPM to a matrix that resembled the sample matrix. Polyamines in tissue, plasma, and urine were quantitated with calibration standards that were prepared in 0.6 N perchloric acid, control plasma, and control urine, respectively. The concentration of each polyamine was calculated from the peak area by calibration curves obtained by nonweighted least squares linear regression. Peak area and linear regression calculations were performed on a Macintosh Centris 650 with Rainin Dynamax HPLC Method Manager software (Rainin Instrument Co., Ridgefield, NJ). The method had a detection limit of 1 nmol/ml, and was reproducible and linear over a range of 1–100 nmol/ml. Plasma and urine samples were prepared for HPLC analysis by the procedure we have previously published (7).

Pharmacokinetic Analyses. Model-independent and model-dependent pharmacokinetic parameters were estimated from DEHSPM plasma concentration-time data with the aid of PCNONLIN (SCI Software, Lexington, KY). The maximum plasma concentrations were read from each individual time-concentration curve. The k_{el} was estimated by linear regression of the terminal concentration-time data; the $t_{1/2}$ was calculated from $0.693/k_{el}$. The AUC from time 0 to the time of the last measurable plasma concentration (6 hr) was calculated by the trapezoidal method. $AUC_{0-\infty}$ was extrapolated by adding $AUC_{0-6\ hr}$ to the quotient of C_p/k_{el}, where C_p is the last measured concentration (*i.e.* at t = 6 hr). V_d was calculated as CL/k_{el}, and CL was calculated from Dose/$AUC_{0-\infty}$. Values for CL and V_d were normalized to adjust for differences in body weights. MRT was obtained from $AUMC_{0-\infty}/AUC_{0-\infty}$, where AUMC is the total area under the first-moment curve ($C_p{*}t$ *vs.* t). Renal clearance was calculated as U_{0-4}/AUC_{0-4}, where U_{0-4} is the amount of DEHSPM excreted in urine over a 4-hr collection interval (0–4 hr) divided by the plasma AUC of DEHSPM over the same interval.

Results

Acute and Chronic Toxicity of DEHSPM and Its Metabolites in Mice. The acute toxicity of DEHSPM and its deethylated metabolite, HSPM, is essentially identical to the corresponding NSPM analogs, DENSPM and NSPM (fig. 1). The bis-primary amines, HSPM and NSPM, are somewhat more toxic when administered systemically than the *N*-ethyl homologs, probably because they can be converted to toxic aldehydes by widely distributed, nonspecific amine oxidases (8, 9). There are, however, impressive differences in the toxicities of the HSPM analogs in comparison with the corresponding NSPM compounds when administered in a chronic dosage regimen consisting of intraperitoneal injections every 8 hr for 6 days (18 doses in total). The chronic LD_{50} for DEHSPM is estimated at ~13.3 mg/kg tid (total of 40 mg/kg/day), compared with 67 mg/kg tid for DENSPM (total of 200 mg/kg/day) (fig. 2). The chronic LD_{50} for HSPM is ~8 mg/kg tid, whereas no deaths were observed in the NSPM-treated groups at doses up to 30 mg/kg tid, although at this dose the mice had lost

TABLE 1

Accumulation of DEHSPM and metabolites in mouse liver and kidney

Tissue	Day	% Total Dose[a] Present in Tissue[b] as				% Total in Tissue DEHSPM + Metabolites	% Total in Both Tissues		
		DEHSPM	±SD	Metabolites	±SD		DEHSPM	Metabolites	DEHSPM + Metabolites
Liver	2	3.9	0.3	6.5	1.0	10.4	15.3	17.3	32.6
	4	4.8	1.0	13.0	1.9	17.8	12.1	22.7	34.8
	7	4.9	0.5	17.4	1.0	22.3	9.6	25.5	35.1
Kidney	2	11.4	1.2	10.8	0.7	22.1			
	4	7.3	1.0	9.7	0.8	17.0			
	7	4.7	0.2	8.1	0.2	12.8			

[a] Total Dose = 10 mg/kg/day. → 0.6 mg for a 30 g mouse at 2 days; 1.2 mg at 4 days; and 2.1 mg at 7 days.

[b] Total amount present in each tissue is calculated from the content/g wet wt. tissue (fig. 3) and the weight of that tissue normalized for a 30 g mouse: liver = 2.623 g and kidney = 0.480 g (see ref. 10).

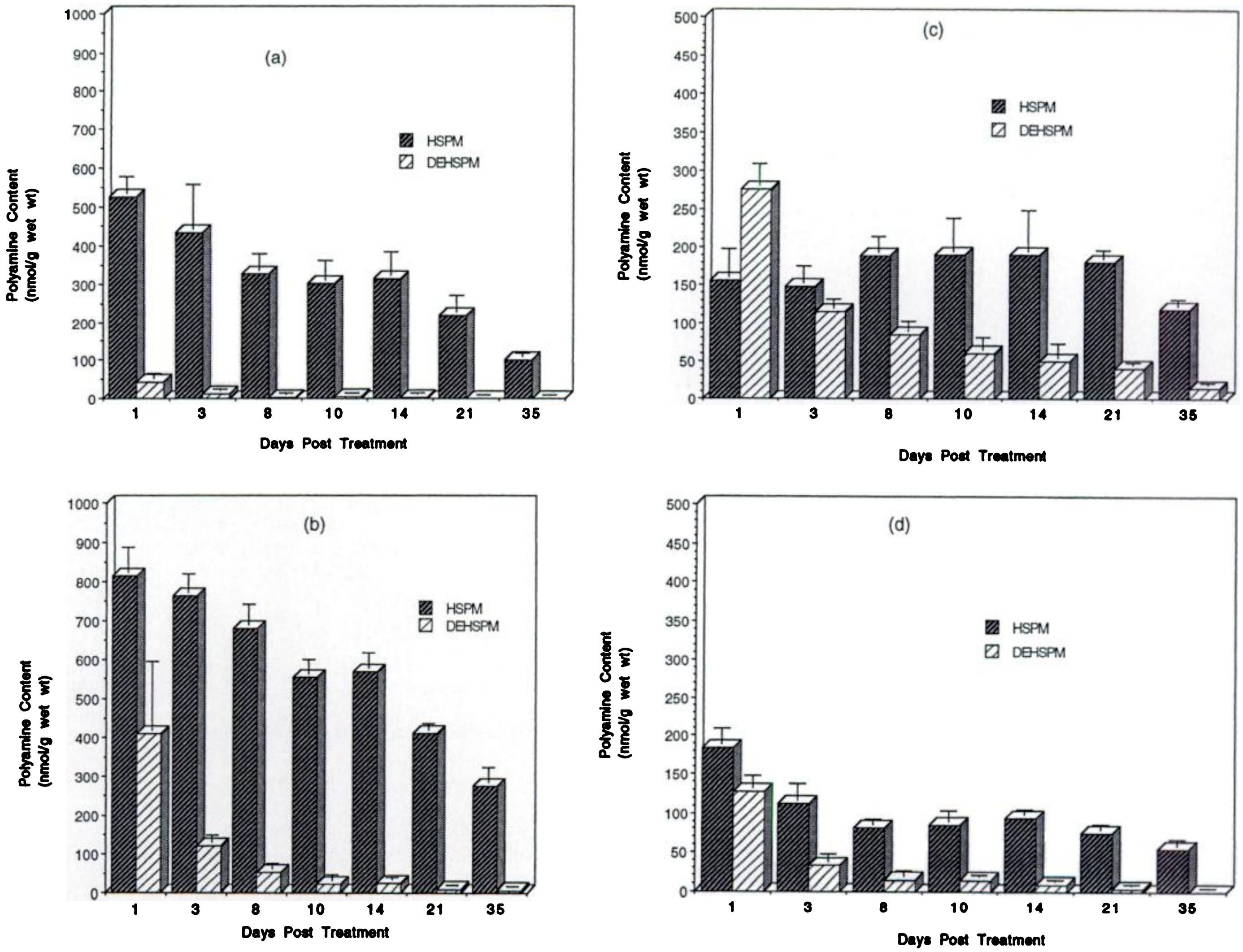

FIG. 4. *Disappearance of DEHSPM and persistence of the di-deethylated tetraamine metabolite, HSPM, in mouse tissues: (a) liver, (b) kidney, (c) spleen, and (d) small intestine.*

Mice were treated with once daily intraperitoneal injections of 15 mg/kg/day DEHSPM for 6 days. Groups of mice (N = 3; mean ± SD) were killed for analysis on days 1, 3, 8, 10, 14, 21, and 35 after the final DEHSPM treatment.

~10% of body weight at the time of final treatment. Although animals treated with DENSPM always died within 1–2 days of the final treatment and the condition (body wt., coat appearance, etc.) of survivors rapidly improves during the week after the final treatment, DEHSPM-treated mice judged as survivors 1 day after cessation of treatment may nonetheless die up to 7 days later. This phenomenon was most pronounced in the HSPM-treated groups. One day after the final dose, all animals in all three dosage groups were alive. However, on days 3–4 posttreatment, 3 of the 15 mg/kg tid group died; by day 6, 2 of the 10 mg/kg tid group had died; and by day 10, 5 of 5 of the 15 mg/kg, 3 of 5 of the 10 mg/kg, and 1 of 5 of the 5 mg/kg tid groups had died.

Accumulation of DEHSPM and Metabolites in Mouse Tissues. The rates of accumulation of DEHSPM and its metabolites, as well as the effects on native polyamine pools in mouse liver and kidney, were assessed by treating mice with a single daily dose of 10 mg/kg DEHSPM intraperitoneally for 7 days. Six hr after injection of DEHSPM on days 2, 4, and 7, animals were killed for tissue analysis. The level of DEHSPM in the liver is similar at each time (fig. 3*a*) and represents 4–5% of the total drug administered (table 1). The monodeethylated metabolite, MEHSPM, is present at about equal levels at each time in an amount ~10% that of DEHSPM. The dideethylated metabolite, HSPM, is present at higher levels than DEHSPM even at 2 days and in progressively larger amounts at later times, accounting for ~6%, 12.5%, and 17% of the total dose of DEHSPM on days 2, 4, and 7, respectively. The liver seems to maintain a nearly constant level of total polyamine nitrogen equivalents (fig. 3*a*), so that the increase of [DEHSPM + metabolites] is balanced by a concomitant decrease in content of the native polyamines, SPM and SPD.

The levels of DEHSPM in the kidney are slightly higher than those of HSPM on day 2 (fig. 3*b*). Although [DEHSPM] continues to increase, [HSPM] increases to an even greater degree. As in the liver, MEHSPM is present at comparatively low levels (20–37 nmol/g wet wt.). A progressive decrease in SPM is the prominent effect on native polyamines, so that HSPM represents the major polyamine in the kidney by day 4. By day 7, SPM has declined to 405 nmol/g wet wt. from the control ("day 0") value of 830 nmol/g wet wt., whereas HSPM has risen to 780 nmol/g wet wt. on day 7.

Analysis of these data in terms of the fate of the total amount of DEHSPM administered to the animal reveals several noteworthy

TABLE 2

Persistence of DEHSPM and metabolites in the mouse liver, kidney, and spleen

Tissue	Day	% Total Dose[a] Present in Tissue[b] as				% Total in Tissue Drug + Metabolites	% Total in All Three Tissues as		
		DEHSPM	±SD	Metabolites	±SD		DEHSPM	Metabolites	Drug + Metabolites
Liver	1	1.8	0.5	22.3	2.1	24.1	5.6	29.3	34.9
	3	0.5	0.1	18.3	4.9	18.8	1.7	24.6	26.3
	8	0.1	0.2	13.7	1.9	13.8	0.7	19.5	20.2
	10	0.1	0.0	13.6	2.1	13.8	0.5	18.6	19.1
	14	0.2	0.0	13.2	2.6	13.3	0.4	18.1	18.5
	21	0.0	0.0	9.2	1.8	9.2	0.2	12.8	13.0
	35	0.0	0.0	4.2	0.3	4.2	0.1	6.6	6.7
Kidney	1	3.1	1.4	6.5	0.7	9.7			
	3	0.9	0.1	5.9	0.4	6.8			
	8	0.4	0.1	5.3	0.4	5.7			
	10	0.2	0.1	4.5	0.5	4.7			
	14	0.2	0.1	4.4	0.6	4.6			
	21	0.1	0.0	3.1	0.3	3.2			
	35	0.0	0.0	2.1	0.3	2.1			
Spleen	1	0.7	0.1	0.5	0.1	1.1			
	3	0.3	0.0	0.4	0.1	0.7			
	8	0.2	0.0	0.5	0.1	0.7			
	10	0.1	0.0	0.5	0.1	0.6			
	14	0.1	0.0	0.5	0.1	0.6			
	21	0.1	0.0	0.5	0.0	0.6			
	35	0.0	0.0	0.3	0.0	0.3			

[a] Total Dose = 15 mg/kg/day × 6 days → 2.7 mg for a 30 g mouse.

[b] Total amount present in each tissue is calculated from the content/g wet wt. tissue (fig. 4) and the weight of that tissue normalized for a 30 g mouse; liver = 2.623 g; kidney = 0.48 g; and spleen = 0.15 g (see ref. 10).

features (table 1). Slightly more than one-third (32.6–35.1%) of the total dose is present in the liver and kidneys as DEHSPM and HSPM, with MEHSPM making a minor contribution. Although this amount remains essentially constant from days 2 to 7, the relative proportion of metabolites to DEHSPM progressively increases from 53% metabolites on day 2 to 72.6% metabolites on day 7. The relative distribution of the dose between the two tissues also changes. Whereas 22.1% of the total dose is present in the kidneys and 10.4% in the liver at day 2, subsequent doses of drug seem to be selectively accumulated in the liver, so that by day 7 the liver contains 22.3% of the total dose, with 12.8% in the kidneys. This may reflect a comparative saturation of the kidneys to exogenous polyamines, because the internal pools, especially SPM (fig. 3*b*), have been substantially depleted, and the total polyamine [*N*+] equivalents already exceed control by 35% on day 2 and grow to 64.7% at day 7. Thus, although the kidneys do continue to accumulate significant quantities of DEHSPM from doses administered after day 2, it is clear that polyamine homeostasis in this tissue has been substantially disrupted in comparison with the liver.

Disappearance (Washout) of DEHSPM and Metabolites in Mouse Tissues. In this experiment, mice were administered a single daily dose of 15 mg/kg DEHSPM ip for 6 days. Groups of three of these animals were then killed 1, 3, 8, 10, 14, 21, and 35 days after the final DEHSPM treatment, and the tissues were analyzed by HPLC to assess the rates of disappearance or washout of DEHSPM and its metabolite(s). This treatment regimen produced essentially an identical result on day 1 after cessation of treatment, as was observed on the final day of the accumulation experiment discussed herein. The day 1 liver and kidneys (fig. 4, *a* and *b*) contained almost identical amounts of DEHSPM and HSPM, as observed on the final day of the accumulation experiment (fig. 3), and these two tissues accounted for 34.9% (table 2) or 35.1% (table 1) of the total dose of DEHSPM administered in these respective experiments.

The levels of DEHSPM and its major metabolite, HSPM, are plotted for the liver, kidney, spleen, and small intestine (fig. 4, *a–d*, respectively) at the specified times from 1 to 35 days after the final DEHSPM treatment. The monodeethylated metabolite, MEHSPM, is present at levels generally ~10% those of DEHSPM and are not included in these plots for clarity. The relative proportion of DEHSPM to HSPM present on day 1 after cessation of treatment varies, depending on the tissue (table 2). At this time, almost one-fourth of the total dose was present in the liver as DEHSPM and its tetraamine metabolites. About 90% of the liver content was HSPM, whereas <10% was present as DEHSPM 1 day posttreatment. The remaining liver DEHSPM essentially disappears over the next week, with an apparent $t_{1/2}$ = 1.6 days for DEHSPM, and the tissue half-life of MEHSPM in the liver is of a similar order of magnitude (~1–1.5 days). By comparison, HSPM persists in the tissue for a remarkably long time, with an estimated $t_{1/2}$ = 15.4 days in mouse liver (fig. 4*a*).

The kidney contained the highest concentrations of DEHSPM and HSPM of any tissue analyzed (~400 and 800 nmol/g wet wt., respectively) on day 1. However, because of the smaller organ weight of the kidneys compared with the liver, on day 1, the former organ contains ~10% of the total DEHSPM administered, compared with almost 25% in the liver. As in the liver, DEHSPM ($t_{1/2}$ = 2 days) levels decline comparatively rapidly in the kidney, compared with HSPM ($t_{1/2}$ = 21.6 days). A qualitatively similar pattern (*i.e.* DEHSPM $t_{1/2}$ = 2–4 days and HSPM $t_{1/2}$ > 22 days) was observed in the spleen and small intestine (fig. 4, *c* and *d*). The practical consequence is that within 1 week after cessation of DEHSPM treatment, very little

TABLE 3

Metabolic fate of DEHSPM in the liver, kidney, spleen, and lung of dogs receiving chronic (21 or 29 days) twice daily subcutaneous injections

	Dosage[a]–Days Posttreatment	Mole Fraction[b] of Tetraamine Metabolite			Total Tetraamine Metabolites	% Total Dose[c] Present in Tissue[a] as			% Total in All Four Tissues[a] as		
		HSPM	MEHSPM	DEHSPM		DEHSPM	Metabolites	DEHSPM + Metabolites	DEHSPM	Metabolites	DEHSPM + Metabolites
					nmol/g wet wt.						
Liver	1 mg/kg–3 days	0.439	0.286	0.275	636	8.3	22.0	30.3	11.8	24.9	36.6
	1 mg/kg–10 days	0.828	0.086	0.086	582	2.4	25.4	27.8	4.4	29.2	33.6
	2 mg/kg–3 days	0.675	0.196	0.129	961	3.0	20.0	22.9	6.4	23.2	29.6
	2 mg/kg–10 days	0.832	0.080	0.089	903	1.9	19.6	21.5	4.0	23.9	27.9
	4 mg/kg–3 days	0.732	0.129	0.139	1109	2.5	15.7	18.3	5.8	19.1	24.9
	4 mg/kg–10 days	0.876	0.065	0.059	1419	1.4	22.0	23.4	3.5	26.0	29.5
Kidney	1 mg/kg–3 days	0.509	0.193	0.298	285	0.6	1.5	2.1			
	1 mg/kg–10 days	0.755	0.105	0.141	220	0.2	1.4	1.6			
	2 mg/kg–3 days	0.501	0.199	0.300	417	0.5	1.1	1.6			
	2 mg/kg–10 days	0.604	0.175	0.220	445	0.4	1.3	1.7			
	4 mg/kg–3 days	0.551	0.156	0.293	788	0.6	1.4	2.0			
	4 mg/kg–10 days	0.695	0.114	0.192	731	0.4	1.5	1.9			
Spleen	1 mg/kg–3 days	0.134	0.077	0.789	209	0.6	0.2	0.8			
	1 mg/kg–10 days	0.311	0.104	0.585	135	0.3	0.2	0.5			
	2 mg/kg–3 days	0.136	0.084	0.780	545	0.8	0.2	1.0			
	2 mg/kg–10 days	0.315	0.147	0.537	428	0.4	0.4	0.8			
	4 mg/kg–3 days	0.225	0.087	0.688	952	0.8	0.4	1.2			
	4 mg/kg–10 days	0.389	0.110	0.501	709	0.5	0.5	0.9			
Lung	1 mg/kg–3 days	0.133	0.226	0.642	226	2.2	1.2	3.4			
	1 mg/kg–10 days	0.335	0.261	0.404	245	1.5	2.2	3.7			
	2 mg/kg–8 days	0.217	0.261	0.522	552	2.1	2.0	4.1			
	2 mg/kg–10 days	0.387	0.282	0.330	524	1.3	2.6	3.9			
	4 mg/kg–3 days	0.246	0.209	0.545	659	1.8	1.5	3.4			
	4 mg/kg–10 days	0.381	0.233	0.387	649	1.3	2.0	3.3			

[a] Total amount present in each tissue is calculated from the content/g wet wt. tissue (fig. 5) and the weight of that tissue normalized for a 10 kg dog: liver = 320 g; kidney = 50 g; spleen = 25 g; and lung = 100 g (see ref. 10).

[b] Mole fraction of tetraamine metabolites of DEHSPM, wherein HSPM + MEHSPM + DEHSPM = 1.000.

[c] Total Dose for a 10 kg dog → 1 mg/kg/day × 29 days = 290 mg; 2 mg/kg/day × 29 days = 580 mg; and 4 mg/kg/day × 21 days = 840 mg. One dog from each dosage group was killed 3 days and the other 10 days after final treatment.

unmetabolized DEHSPM remains in tissues, whereas a substantial portion of the original dose persists in the form of HSPM. For example, on posttreatment day 8, 20.2% of the original DEHSPM dose remains in the liver, kidney, and spleen: 19.5% as HSPM and only 0.7% as DEHSPM. It is remarkable that even 35 days after cessation of treatment, 6.6% of the total DEHSPM administered is present in these three tissues, but almost exclusively in the form of HSPM (table 2).

Toxicity and Tissue Distribution of DEHSPM and Metabolites in Dogs. Based on pharmacokinetic analysis of DEHSPM administered subcutaneously (see herein), two subcutaneous injections/day should provide a significant plasma level of drug almost continuously. Three experimental groups of two dogs received this subcutaneous DEHSPM treatment regimen at doses of 1 or 2 mg/kg/day for 29 days, or 4 mg/kg/day for 21 days, respectively. The dogs were killed either 3 or 10 days after the final DEHSPM treatment, and the tissues were analyzed for DEHSPM and metabolites by HPLC. The dogs were carefully monitored for signs of toxicity during the study. The animals in the two lower dose groups, 1 or 2 mg/kg/day, were bright, alert, and responsive at the beginning of the study and remained so throughout the course of the experiment. The animals in the 4 mg/kg/day group were also bright, alert, and responsive for the first 2 weeks of the study, but their activity level and general appearance deteriorated significantly during the third week.

Blood samples were taken twice a week, whereas urine samples were obtained once a week. The only significant findings were in the 4 mg/kg/day group. Both of these animals had elevated SGOT enzymes by the end of the third week, suggesting liver toxicity. In addition, one of the animals had an elevated white blood cell count. There were no other remarkable findings in any of the other blood chemistries or urinalysis.

The study was stopped after 21 days in the 4 mg/kg/day group because of anorexia, decreased activity, and bloody diarrhea. These animals were killed 3 and 10 days after the final DEHSPM treatment. It is notable that the condition of both animals continued to deteriorate even after cessation of treatment and in spite of attempts to improve nutrition by switching to canned dog food and providing Nutrical caloric supplement.

Tissue samples were obtained at necropsy from all animals except one of the high dose group and were examined for evidence of histopathological changes, which were observed in the liver of the 2 and 4 mg/kg/day groups. It is of interest that only the animal that was killed 10 days after the final 2 mg/kg/day treatment showed diffuse, moderate-to-severe centrilobular hepatocyte vacuolation. The other 2

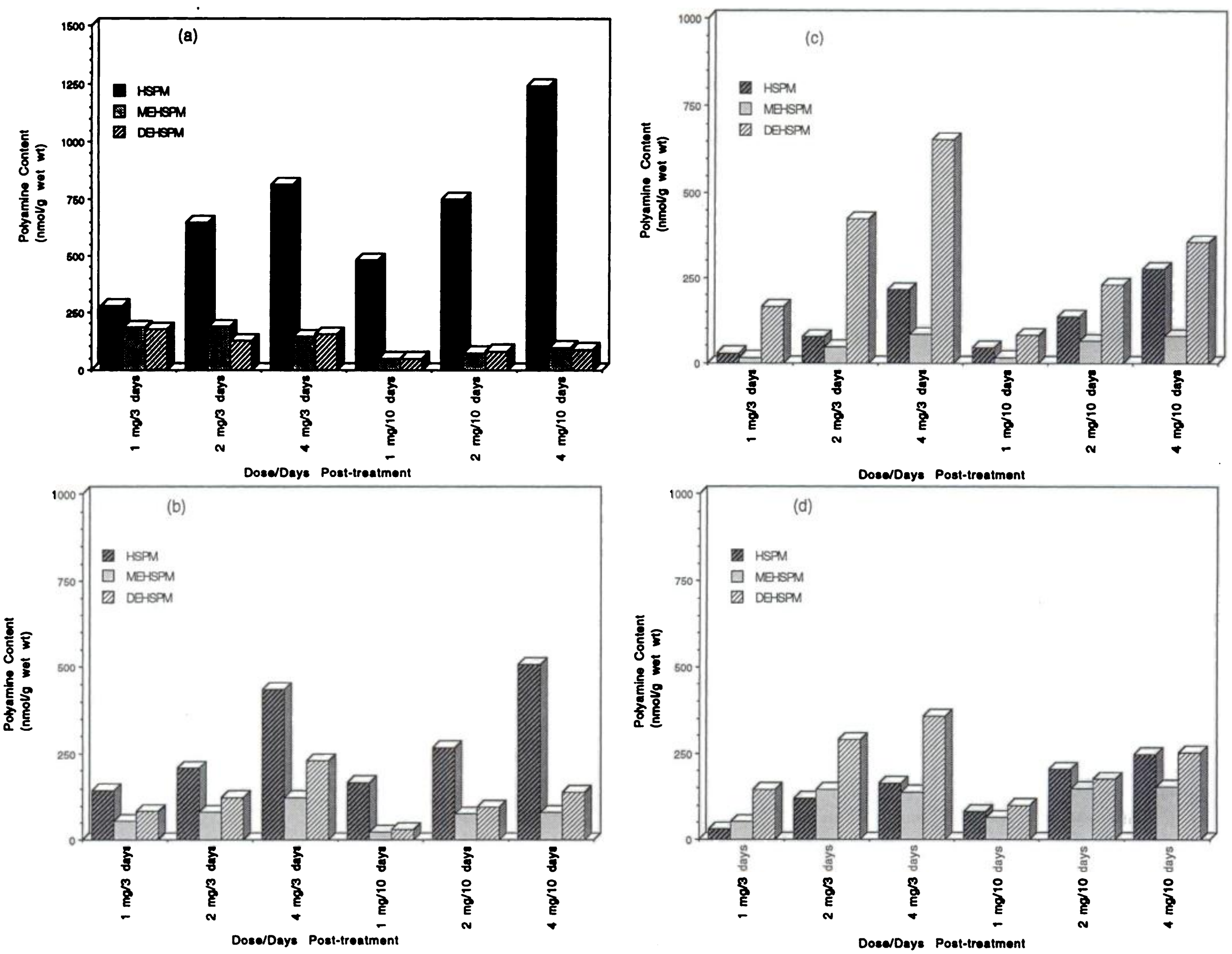

FIG. 5. *Tissue concentrations of DEHSPM and tetraamine metabolites, MEHSPM and HSPM in dog tissues: (a) liver, (b) kidney, (c) spleen, and (d) lung.*

Six dogs were divided into three groups of two each and treated chronically with twice daily subcutaneous injections of isotonic DEHSPM according to one of the following dosage regimens: 1 mg/kg/day × 29 days; 2 mg/kg/day × 29 days; and 4 mg/kg/day × 21 days. One animal from each dosage group was killed 3 days after the final dose (plotted on the *left side* of the graph), and the other animal from each group was killed 10 days after the final dose (plotted on the *right side* of the graph).

mg/kg/day animal that was killed on posttreatment day 3 showed some very mild changes that may have been an early stage of pathological change, but was judged to be negative. The 10-day posttreatment animal in the 4 mg/kg/day group showed moderate-to-severe histopathology in the liver and kidneys, as well as significant changes in the spleen and large and small intestinal mucosa. Thus, the most affected tissues correspond to the tissues with the highest concentrations of DEHSPM and its metabolites, namely the liver and kidney (table 3). Moreover, the observations in the liver of the 2 mg/kg/day dogs suggest that the time the tissue remains exposed to moderately high levels of these tetraamines (*i.e.* Σ[HSPM+MEHSPM+DEHSPM]) may also be of importance. The role of exposure time in the development of toxicity becomes a critical issue, given the persistence of HSPM in both the liver and kidney (fig. 5 and table 3). In many respects, the distribution and tissue levels of DEHSPM and metabolites are similar in dogs and mice. The liver contained between 18 and 30% of the total dose of DEHSPM, and HSPM was the predominant metabolite in all cases. In the liver and kidney, the level of both DEHSPM and MEHSPM is lower in the dogs killed 10 days after cessation of DEHSPM treatment when compared with the corresponding posttreatment day 3 animals. In each dosage group, the HSPM level is highest in the 10-day posttreatment animal, although the total tissue content of all three HSPM tetraamines (expressed as nmol/g wet wt.) is similar at 3 and 10 days posttreatment (*i.e.* $\Sigma[\text{HSPM+MEHSPM+DEHSPM}]_{3\ \text{days}} = \Sigma[\text{HSPM+MEHSPM+DEHSPM}]_{10\ \text{days}}$) for each dosage group in the liver and kidney (table 3). Moreover, there seems to be a cumulative dose effect with respect to total tissue content of DEHSPM + metabolites (compare "total" tissue levels *vs.* total dose in table 3). Both in mice and dogs, DEHSPM is metabolized as depicted in fig. 6. After two *N*-deethylations, DEHSPM is converted to HSPM, which is not recognized by the constituitive polyamine degradative pathways, and therefore accumulates to toxic levels.

Pharmacokinetics of DEHSPM in Dogs. Two short-term (15 min) and one 2-hr constant rate intravenous infusion experiments (fig. 7 and table 4) gave results that were similar to results we have previously reported for the closely related drug, DENSPM (7). As expected, these infusions essentially saturated the distributive phase of

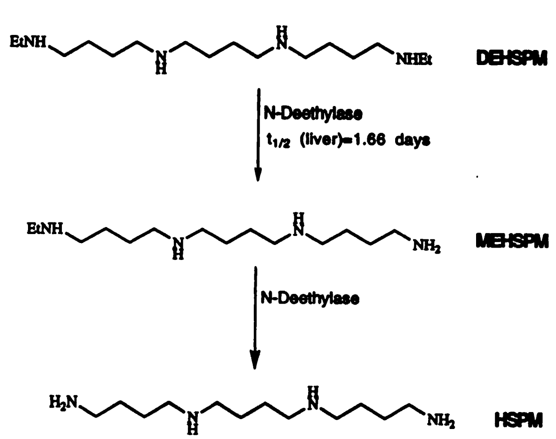

FIG. 6. *Metabolism of DEHSPM.*

Because there are no constituitive degradation pathways to remove aminobutyl equivalents, HSPM accumulates ($t_{1/2}$ > 15–20 days). Et, ethyl.

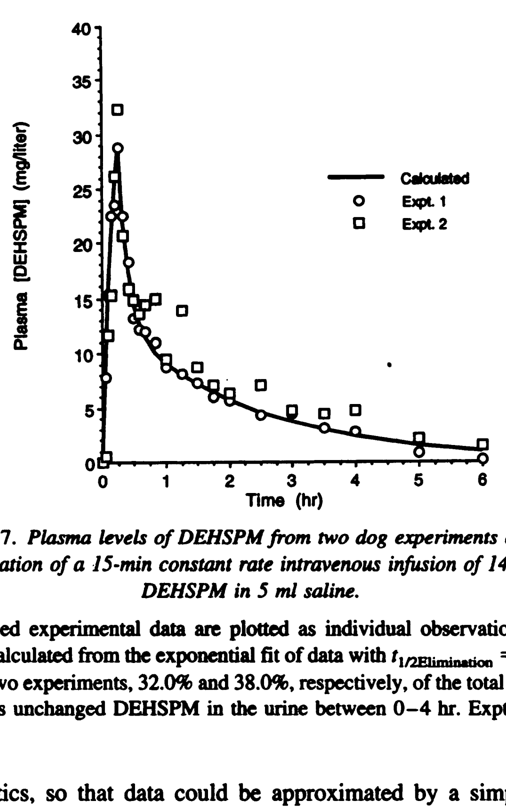

FIG. 7. *Plasma levels of DEHSPM from two dog experiments after administration of a 15-min constant rate intravenous infusion of 14.8 mg/kg DEHSPM in 5 ml saline.*

Observed experimental data are plotted as individual observations. *Solid curve* is calculated from the exponential fit of data with $t_{1/2\text{Elimination}}$ = 1.04 hr. In these two experiments, 32.0% and 38.0%, respectively, of the total dose was isolated as unchanged DEHSPM in the urine between 0–4 hr. Expt., experiment.

the kinetics, so that data could be approximated by a simple one-compartment model with elimination half-lives estimated as 1.07, 1.01, and 1.88 hr, respectively. The apparent V_d's (table 4) were small (0.519 and 0.508 liter/kg), as might be expected for such a highly charged water-soluble molecule. The renal excretion data showed that about one-third of the dose (32%, 38%, and 39.8%) had been excreted as unchanged DEHSPM in the urine during the first 4 hr after treatment. The half-lives from renal excretion data were calculated to be 0.98, 0.99, and 1.13 hr, respectively. The fact that virtually no DEHSPM metabolites were detected in the urine was remarkable in view of the presence of substantial amounts of such metabolites, especially the fully deethylated metabolite, HSPM, in all tissues examined.

In two experiments, the drug was administered subcutaneously in saline to evaluate this route of administration as a slow release depot able to sustain blood levels for more extended periods of time, compared with a short-term intravenous infusion dose (MRT = 4.66 and 4.67 hr, compared with the 15-min intravenous infusion values of MRT = 1.81 and 2.04 hr). Moreover, subcutaneous administration produced peak plasma DEHSPM values of ~5 mg/liter, which were sustained at nearly a constant level for the next 4 hr before slowly tapering off to "0" at 10–12 hr (fig. 8). As in the cases of intravenous administration, urine contents mirror plasma levels with 26.4 and 22.8% of the total dose excreted unchanged in the urine during the first 4 hr.

Discussion

The biochemical machinery for metabolism of the native polyamines SPM, SPD, and PUT is expressed in almost all cells in the body. The key polyamine biosynthetic enzymes, ornithine decarboxylase and AdoMetDC are tightly regulated proteins with short half-lives and high turnover. SPM can be converted back to SPD and then to PUT by the catabolic cytosolic enzymes SSAT and the flavoprotein, PAO. SSAT transfers an acetyl from acetyl coenzyme A to the primary amino (N^1) terminus of 3-aminopropyl moieties of SPM, SPD, or other polyamine substrates (11, 12). *SSAT does not act on the aminobutyl half (N^8) of SPD or PUT (N^1,N^4-Diaminobutane) itself.* The N^1-acetyl derivatives are regiospecifically oxidized by PAO at the adjacent internal secondary nitrogen to yield SPD from N^1-acetylspermine and PUT from N^1-acetylspermidine, together with 3-acetamidopropanal in both instances. This aldehyde is further oxidized to N-acetyl-β-alanine, which is hydrolyzed to β-alanine. Thus, the aminobutyl moiety from PUT is conserved in the polyamine metabolic cycle, whereas the aminopropyl moieties originating from methionine *via* AdoMetDC are irreversibly eliminated as β-alanine.

We have reported that SSAT/PAO is a critical pathway in the metabolism of the antineoplastic drug, DENSPM (7). DENSPM is comprised of three aminopropyl moieties, so that once terminal N-deethylation occurs, the NSPM backbone is completely degraded. Small quantities of each of the expected amine metabolites (MENSPM, NSPM, N^1-ethylnorspermidine, norspermidine, N^1-ethyl-1,3-diaminopropane, and 1,3-diaminopropane) are detected in the liver, kidney, spleen, and lung tissues of both the mouse and dog. The key observation is that *there is no accumulation of toxicologically significant levels of metabolites in animals treated with DENSPM.* For example, in an experiment analogous to the washout experiment, we treated mice with 60 mg/kg DENSPM once a day for 6 days (see table 2 in ref. 7). Twelve hr after cessation of treatment, the liver contained ~5% of the total dose as DENSPM, 1.9% as MENSPM, only 0.25% as NSPM, and ~0.2% as triamine and diamine metabolites. By posttreatment day 4, only 1.0% of the total dose remains in the liver as DENSPM ($t_{1/2}$ = 1.5 days), 1.4% as MENSPM, 0.4% as NSPM, and 0.1% as triamine and diamine metabolites. These DENSPM data compare with the following DEHSPM data for mouse liver (table 2): day 1—1.8% as DEHSPM, 0.3% as MEHSPM, and 22% as HSPM; and day 3—0.5% as DEHSPM, <0.1% as MEHSPM, and 18.2% as HSPM. Even 35 days after the final DEHSPM treatment, mouse liver contains 4.2% of the total drug administered, and it is all in the form of HSPM. Thus, the ultimate metabolic disposition of DEHSPM is dramatically different from that of DENSPM. The initial N-deethylation steps are identical, with a similar $t_{1/2}$ = 1.5–1.6 days, resulting first in MEHSPM or MENSPM and then HSPM or NSPM, respectively. However, HSPM is comprised of three aminobutyl moieties and thus is not recognized as a substrate for the SSAT/PAO degradative pathway (11). HSPM is in effect the metabolic endproduct of DEHSPM metabolism and accumulates in the cell because of lack of effective catabolic control.

TABLE 4

Pharmacokinetic parameters determined in two dogs from plasma levels and urinary excretion after intravenous administration of 14.8 mg/kg DEHSPM

Parameter	Method of Administration		
	15-Min Infusion	2-Hr Infusion	Subcutaneous
Dose (mg)	147.7/150.7	158.4	158.4/151
Body weight (kg)	10.0/10.2	10.7	10.7/10.2
t_{max} (hr)	0.25/0.25	2.0	~1.5–5.0
C_{max} (mg/liter)	28.9/32.3	13.1	~5
AUC_{0-4} (mg/hr/liter)	38.8/35.6	33.2	18.1/14.5
$AUC_{0-\infty}$ (mg/hr/liter)	44.0/42.3	51.4	43.3/32.8
$AUMC_{0-\infty}$ (mg/hr^2/liter)	79.54/86.2	193.9	201.7/153.1
CL (liter/hr/kg)	0.336/0.349	0.288	0.342–0.451
CL_R (liter/hr/kg)	0.122/0.158	0.177	0.216/0.233
MRT (hr)	1.81/2.04	3.77	4.66/4.67
$t_{1/2}$ (hr)			
Elimination[a]	1.07/1.01	1.88	—
Urinary[b]	0.98/0.99	1.13	—
V_d (liter/kg)	0.519/0.508	—	—
Cumulative urinary excretion (0–4 hr) (% of dose)	32.0/38.0	39.8	26.4/22.8

C_{max}, maximum concentration; AUMC, area under the first-moment curve; CL_R, renal clearance.

[a] Half-life evaluated from plasma concentration data.

[b] Half-life evaluated from urinary excretion data.

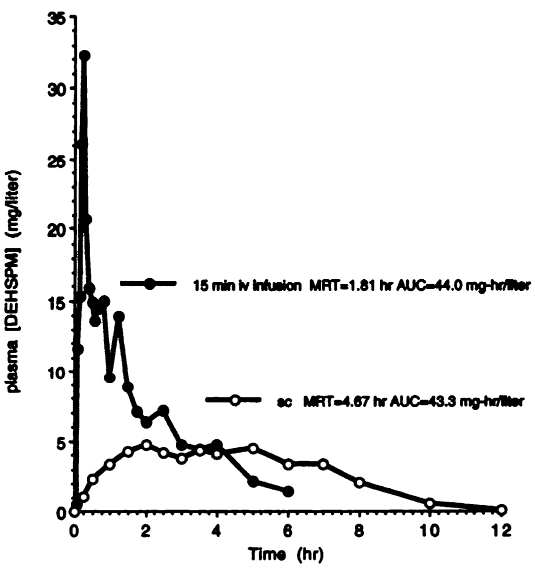

FIG. 8. *Subcutaneous injection (solid line) vs. 15-min constant rate intravenous infusion (dashed line) of 14.8 mg/kg DEHSPM in two dog experiments.*

Plasma concentration (mg/liter) *vs.* time (hr). Observed plasma data are plotted as *solid circles* for the intravenous experiment and *open circles* for the subcutaneous data. Noncompartmental analysis of these data showed a MRT of 4.67 hr (subcutaneous) *vs.* 1.81 hr (intravenous). A comparison of the AUCs for these two experiments [43.3 mg/hr/liter (subcutaneous) *vs.* 44.0 mg/hr/liter (intravenous)] suggests a very high (98%) bioavailability of DEHSPM administered by the subcutaneous route. A comparison of two other similar experiments (not shown) shows AUCs of 32.8 mg/hr/liter (subcutaneous) *vs.* 42.3 mg/hr/liter (intravenous) or 78% bioavailability of the subcutaneous dose of DEHSPM and MRTs of 4.66 hr (subcutaneous) *vs.* 2.04 hr (intravenous).

The plasma pharmacokinetics of DEHSPM and DENSPM (7) are similar to each other and are as expected for highly water-soluble, lipid-insoluble molecules. Certainly, these similar plasma pharmacokinetics cannot explain the 4- to 5-fold greater chronic toxicity of DEHSPM in comparison with DENSPM. A significant portion of the DEHSPM dose is shown to be concentrated in particular body tissues, especially the liver and kidney, where it remains for extended periods of time. These two organs are shown to exhibit toxicologically significant changes in dogs receiving DEHSPM chronically. DEHSPM is metabolized by *N*-deethylation to MEHSPM and then to HSPM. In contrast to DENSPM, however, HSPM is not further metabolized and therefore accumulates in cells where it remains for extended periods of time with the tissue $t_{1/2}$ estimated to be 15–20 days in the mouse liver and kidney. Even 35 days after cessation of treatment, 6.4% of the total dose remains in the liver and kidney almost exclusively as HSPM and mostly in the liver. Thus, accumulation and retention of HSPM almost certainly contributes to the increased chronic toxicity of DEHSPM over DENSPM.

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