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Pharmacokinetics and metabolism of the antitumor agent sulfamic acid 1,7-heptanediyl ester (sulfamic acid diester) in the mouse and beagle dog

Joanne I. Brodfuehrer, Tracy J. Wilke, and Garth Powis

Mayo Clinic & Foundation, Department of Pharmacology, 200 First Street, S.W., Rochester, MN 55905, USA

Summary. The pharmacokinetics and metabolism of sulfamic acid diester were studied in the beagle dog and mouse. Elimination of sulfamic acid diester from the plasma and whole blood following i.v. administration at a dose of 193 mg/m^2 was best approximated by a three-compartment model in both species. The compound was relatively rapidly cleared from the plasma, with a plasma beta half-life of 2.3 h and 0.9 h and a gamma half-life of 16 h and 3 h in the dog and the mouse, respectively. Sulfamic acid diester was taken up by blood cells and only slowly eliminated with a whole blood gamma half-life of 42 h in the dog and 32 h in the mouse. When sulfamic acid diester was infused i.v. to mice at 15 mg/kg over 8 h, the clearance for the parent drug was 13.2 ml/min kg from the plasma and 3.3 ml/min kg from the whole blood. Urine collected from mouse and dog contained the parent drug and three metabolic/breakdown products, namely, sulfamic acid 1,7-heptanemonoyl ester, sulfamic acid 3-hydroxyl-1,7-heptanediyl ester, and an unidentified product. Excretion of unchanged drug and products in mouse urine over 8 h accounted for less than 16% of the dose of sulfamic acid diester. Sulfamic acid diester did not react with glutathione in buffer, whole blood, or 100000 *g* rat liver cytosol.

Introduction

Sulfamic acid 1,7-heptanediyl ester (sulfamic acid diester) is the most active of a series of busulfan analogs synthesized in an attempt to develop compounds with a broader spectrum of clinical antitumor activity [12]. The structure of sulfamic acid diester is shown in Fig. 1. Sulfamic acid diester is being developed by the National Cancer Institute (USA) for clinical trial. The compound exhibits antitumor activity when given i.p. in a number of animal tumor models, including murine L1210 and P388 leukemias, B-16 melanoma, and the s.c. implanted MX-1 mammary human xenograft [12]. This study reports the preclinical pharmacokinetics and some breakdown products and metabolites of sulfamic acid diester in the mouse and beagle dog.

Materials and methods

Drugs. Sulfamic acid diester (NSC 329680) was supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute (Bethesda, Md, USA). The compound was formulated immediately prior to administration as a 30 mg/ml solution in 10% ethanol, 40% propylene glycol, and 50% 0.05 *M* sodium phosphate (pH 7.4). The formulated sulfamic acid diester was diluted to the required concentration for i.v. administration with 0.9% NaCl. Sulfamic acid 1,7-hepatanemonoyl ester was prepared from sulfamic acid diester by the method of Paborji et al. [13]. Busulfan was purchased from Sigma Chemical Company (St. Louis, Mo, USA). All other chemicals were reagent grade.

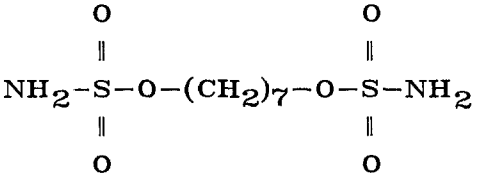

Fig. 1. Structure of sulfamic acid diester

Animal studies. Sulfamic acid diester was administered at a dose of 193 mg/m^2 (45 mg/kg) by rapid i.v. injection (< 30 s) into the tail vein of male CDF$_1$ mice weighing 25–30 g and held in a Broome-type restraint. This dose was the highest tolerated by P388 leukemia tumor-bearing mice [12]. Blood was collected from groups of three mice at 2, 5, 10, 30, 60, 120, 180, 360, 480, and 720 min, and at 18, 24, and 30 h. The times represent the midpoints of the blood collection period that took approximately 30 s. Mice, lightly anesthetized with diethyl ether, were exsanguinated by bleeding from the retro-orbital venous plexus as soon as rapid limb movements had ceased. The blood was collected in chilled heparinized 1.5-ml centrifuge tubes. Part of the blood sample (0.1 ml) was used for measurement of sulfamic acid diester in whole blood. The remaining blood was immediately centrifuged at 10.000 *g* for 2 min and 0.2 ml plasma was taken for analysis of sulfamic acid diester.

Male CDF$_1$ mice weighing 25–30 g were infused through an indwelling PE-10 polyethylene catheter (Intramedic, Clay Adams, Parsipany, NJ) in the tail vein with sulfamic acid diester at a rate of 31 μg/min kg for 8 h, or a total dose of 15 mg/kg. The formulated drug was diluted to 180 μg/ml with 0.9% NaCl and infused at a rate of 5.1 μl/min. Plasma and whole blood were collected from mice killed at 1, 2, 4, 6, and 8 h and subsequently assayed for sulfamic acid diester.

Offprint requests to: G. Powis

Sulfamic acid diester was given to female beagle dogs weighing 15–22 kg by i.v. bolus injection over 1 min at a dose of 193 mg/m^2 (8.5 mg/kg). Dogs were placed in a Pavlov-type sling and the drug was injected through a Teflon catheter (Angiocath 18 gauge, Deseret Co., Sandy, Utah) into a cephalic vein. Samples of blood (3 ml) were drawn into a syringe from a second Teflon catheter in the other cephalic vein at 1, 5, 10, 20, 30, 40, 50, 60, and 90 min, and at 2, 3, 4, 5, 6, 8, 24, 30, 48, 72, 120, and 172 h. The blood was immediately transferred to heparinized tubes. An aliquot of whole blood was removed prior to centrifuging at 3,000 *g* for 2 min to separate plasma. The blood and plasma were frozen at −20°C overnight before assaying for sulfamic acid diester.

Assay. Sulfamic acid diester was assayed by a capillary gas chromatography (GC) method with electron-capture detection following its conversion to 1,7-diiodoheptane as has previously been described [3]. Briefly, 0.2 ml plasma was mixed with 0.2 ml 8 *M* sodium iodide and 0.1 μg busulfan as an internal standard. The mixture was heated in a sealed tube at 70°C for 60 min. After cooling to room temperature, the mixture was shaken for 10 min with 0.8 ml heptane and then centrifuged for 10 min at 3,000 *g*. The upper organic layer was removed and 2 μl taken for GC analysis. The limit of sensitivity of the assay for sulfamic acid diester from 0.2 ml plasma was 50 ng/ml. The breakdown product, sulfamic acid 1,7-heptanemonoyl ester, was identified in the assay by the formation of 7-iodo-1-heptanol.

Pharmacokinetic analysis. Plasma drug concentration data were subjected to nonlinear least-squares regression analysis using the NONLIN pharmacokinetic computer program [11] with a weighting factor of $1/y^2$. Pharmacokinetic parameters were calculated according to the method of Wagner [15].

Urinary excretion. Sulfamic acid diester was administered at a dose of 193 mg/m^2 by rapid i.v. injection into two female beagle dogs and seven mice as described previously. Dogs were housed in stainless steel metabolism cages and urine was collected for 48 h at 4°C. Pooled urine was collected for 8 h at 4°C from seven mice housed in an all-glass metabolism cage. Sulfamic acid diester and its urinary metabolites were measured by the GC assay previously described. Mass spectral analysis of chromatographic peaks of urinary metabolites was carried out with a Kratos MS50 gas chromatograph-mass spectrometer at 70-eV electron impact ionization.

In vitro metabolism. Hepatic microsomes and 100000 *g* cytosol were prepared by differential centrifugation [5] from a homogenate of nonfasted male Fischer 344 rat liver (Sprague-Dawley, Madison, Wis) in 4 volumes of 0.25 *M* sucrose. The microsomes were washed once in 0.15 *M* KCl and suspended in 0.1 *M* sodium phosphate (pH 7.4) at a concentration of 16 mg protein/ml. Protein was assayed by the dye-binding method of Bradford [2] using a commercial test kit (Biorad Laboratories, Richmond, Calif) and crystalline bovine serum albumin as a standard.

Incubations contained 25 mg microsomal protein, 1 mmol sodium-phosphate buffer (pH 7.4), 75 μmol $MgCl_2$, 6 μmol $NADP^+$, 354 μmol glucose-6-phosphate, 105 IU glucose-6-phosphate dehydrogenase, and 1 mg sulfamic acid diester at a volume of 10 ml. In some incubations microsomes heated to 100°C for 10 min were used. The incubation was conducted at 37°C in a slowly reciprocating, 25-ml Erlenmeyer flask open to the air. Samples of the incubation mixture were taken for analysis of sulfamic acid diester and metabolites at 15-min intervals for 2 h.

The reaction between sulfamic acid diester and glutathione was studied by incubating sulfamic acid diester (100 μg/ml) with 0.2 m*M* glutathione in Dulbecco's phosphate-buffered saline at pH 7.4 or pH 8.0 for 1 h. Alternatively, sulfamic acid diester (100 μg/ml) was incubated with fresh dog blood for 1 h. The rate of glutathione disappearance from buffer or whole blood was measured by a HPLC method as previously described [14], in which glutathione was derivatized with monobromobimane to produce a stable, highly fluorescent derivative. Evidence for the enzyme-catalyzed reaction of sulfamic acid diester and glutathione was sought by incubating 12 mg 100,000 *g* rat liver cytosol protein, 1 mmol sodium phosphate buffer (pH 7.4), 10 μmol glutathione, and 1 mg sulfamic acid diester at a volume of 10 ml. For blank incubations cytosol was heated to 100°C for 10 min prior to use. Incubations were conducted at 37°C in slowly reciprocating, 25-ml Erlenmeyer flasks open to the air. Samples of the incubation medium were taken for analysis of both sulfamic acid diester and glutathione at 15-min intervals for 2 h.

Rat hepatocytes were prepared from the livers of male Fischer 344 rats by a low Ca^{2+}, collagenase perfusion technique as has previously been described [1]. The viability of the hepatocytes determined by trypan blue exclusion was routinely greater than 90%. Rat hepatocytes (7×10^7) were incubated in 15 ml Dulbecco's modified Eagles medium containing 10% fetal calf serum under a gas phase of 5% CO_2: 95% air at 37°C in a slowly reciprocating, 50-ml Erlenmeyer flask at 37°C. After 5 min, 1.5 mg sulfamic acid diester was added to start the reaction. Samples of incubation medium were taken at 20-min intervals for 3 h for the analysis of sulfamic acid diester and metabolites.

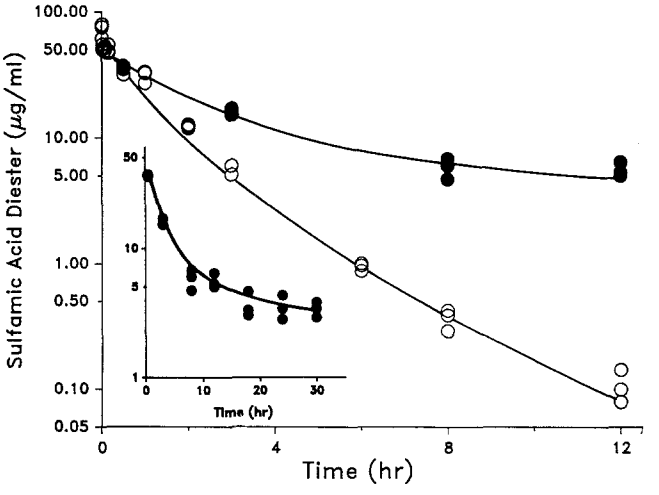

Fig. 2. Plasma (○) and whole blood (●) sulfamic acid diester concentrations in male CDF_1 mice after i.v. bolus administration of 45 mg/kg sulfamic acid diester (193 mg/m^2). *Inset,* whole blood concentrations of sulfamic acid diester over 30 h. Sulfamic acid diester could not be detected in plasma after 12 h. Each point is a single animal; the continuous line is the computer-generated fit to the data

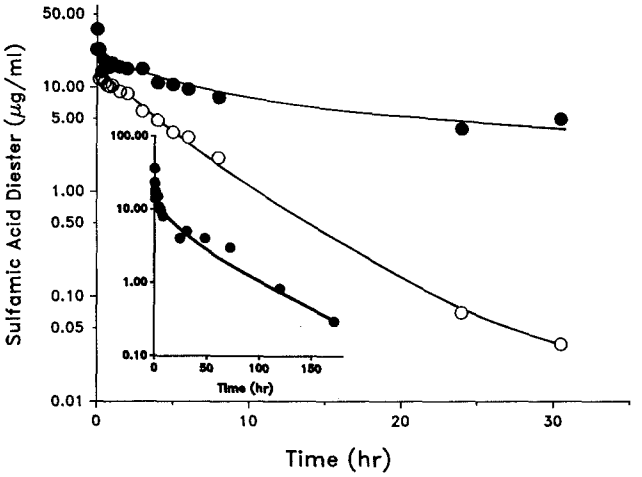

Fig. 3. Sulfamic acid diester plasma (○) and whole blood (●) concentrations in a female beagle dog after i.v. inoculation of 8.5 mg/kg sulfamic acid diester (193 mg/m²). *Inset*, whole blood concentration of sulfamic acid diester over 175 h. Sulfamic acid diester could not be detected in plasma after 30 h. The continuous line is the computer-generated fit to the data

Results

Blood and plasma distribution

Plasma and whole blood concentrations of sulfamic acid diester in mice following an i.v. bolus dose of 193 mg/m² sulfamic acid diester are shown in Fig. 2. The elimination of sulfamic acid diester from the plasma and whole blood was best approximated by a three-compartment open model and the pharmacokinetic parameters are shown in Table 1. Sulfamic acid diester was taken up by the blood cells and unchanged drug could still be detected in the whole blood 30 h after drug administration. In contrast, the plasma concentration of sulfamid acid diester at 18 h after administration had fallen to below the limit of detection for the assay (50 ng/ml). Sulfamic acid diester was also given at an i.v. bolus dose of 193 mg/m² to three female beagle dogs. Elimination of sulfamic acid diester from dog plasma and whole blood was best fit by a three-compartment open model (Fig. 3). Pharmacokinetic parameters for the individual dogs are shown in Table 1. Elimination of sulfamic acid diester from plasma occurred at a slower rate in the dog than in the mouse, with a mean plasma beta half-life of 2.3 h and a gamma half-life of 16 h in the dog, as opposed to 0.9 h and 3 h in the mouse. Sulfamic acid diester was retained by the blood cells in the dog with a mean whole blood gamma half-life of 42 h. A small chromatographic peak at 15.5 min detected by GC assay of mouse and dog plasma was not present in the blank plasma, suggesting some in vivo product formation (Fig. 4). The amount of sulfamic acid 1,7-heptanemonoyl ester in mouse and dog plasma was always $<5\%$ of the amount of sulfamic acid diester, which was consistent with its formation during the assay derivatization procedure [3] and not in vivo.

Plasma and whole blood concentrations of sulfamic acid diester in male CDF_1 mice given sulfamic acid diester by continuous i.v. infusion over 8 h are shown in Fig. 5. Sulfamic acid diester was rapidly taken up by the blood cells, reaching a steady-state concentration in whole blood of 9.6 μg/ml by 2 h. A steady-state plasma concentration

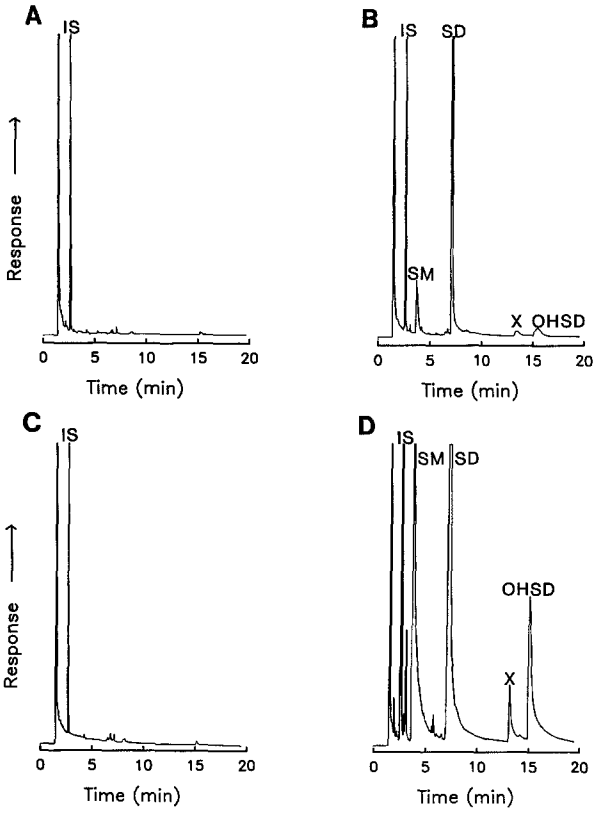

Fig. 4. Chromatograms of sulfamic acid diester in plasma and urine of mice. Sulfamic acid diester was given to mice by i.v. bolus at a dose of 45 mg/kg (193 mg/m²). *A*, Plasma before giving drug; *B*, plasma 3 h after giving drug; *C*, urine before giving drug; *D*, urine collected 8 h after giving drug. Peaks are: *IS*, busulfan internal standard; *SM*, sulfamic acid 1,7-heptanemonoyl ester; *SD*, sulfamic acid diester; *X*, unidentified peak; *OHSD*, 3-hydroxysulfamic acid diester

for sulfamic acid diester of 2.4 μg/ml was achieved within 8 h. The clearance of sulfamic acid diester was 13.2 ml/min kg from the plasma and 3.3 ml/min kg from whole blood. A chromatographic peak at 15.5 min was again detected in the plasma by the GC assay (results not shown).

Table 1. Pharmacokinetic parameters of sulfamic acid diester given by i.v. bolus to mouse and dog

	Mouse	Dog 1	Dog 2	Dog 3
Plasma				
half-life alpha (min)	2.3	0.6	0.6	3.4
half-life beta (h)	0.9	2.9	2.1	1.8
half-life gamma (h)	3.1	ND	19.3	12.8
V_d (ml/kg)	955.7	3147.4	1356.4	1020.4
Cl (ml/min/kg)	10.1	2.4	3.4	4.4
Whole blood				
half-life alpha (min)	ND	3.4	2.1	3.7
half-life beta (h)	1.6	3.1	2.5	3.6
half-life gamma (h)	32.0	37.3	43.6	45.9
V_d (ml/kg)	4430.4	870.1	1095.6	999.7
Cl (ml/min/kg)	2.1	0.3	0.3	0.3

Sulfamic acid diester was given by i.v. bolus to mice and beagle dogs at a dose of 193 mg/m² (45 mg/kg for mouse, 8.5 mg/kg for dog). Pharmacokinetic parameters for sulfamic acid diester in plasma and whole blood were determined from the data in Figs. 2 and 3. V_d, Apparent volume of distribution; Cl, total body plasma clearance; ND, not determined because of insufficient data points

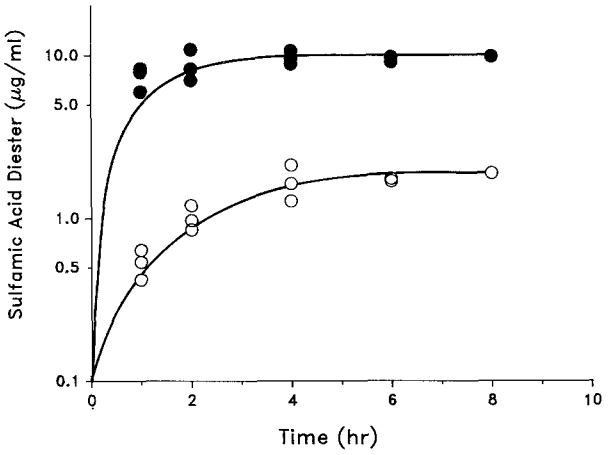

Fig. 5. Plasma (○) and whole blood (●) sulfamic acid diester following i.v. infusion of sulfamic acid diester to male CDF_1 mice at 15 mg/kg over 8 h. Each point represents an animal

Blood was drawn from a dog 120 h after it received sulfamic acid diester and lysed with an equal volume of water. One portion of the lysed blood was dialyzed against water for 48 h at 4° C and a second portion was filtered using an Amicon ultrafiltration cone. There was no loss of sulfamic acid diester from the cellular portion of the blood after dialysis and no sulfamic acid diester was found in the ultrafiltrate. When sulfamic acid diester was mixed with fresh dog blood at 100 μg/mil, there was no decrease in glutathione over 1 h, indicating the absence of an interaction between sulfamic acid diester and blood cell glutathione. The initial glutathione concentration in whole blood was 0.47 m*M* and at the end of the incubation, 0.43 m*M*.

Urinary excretion. Mice excreted an average of 13% of the total dose as unchanged drug in the urine over 8 h, whereas dog 1 and dog 2 excreted 3% and 5%, respectively, of the total dose as unchanged drug in the urine over 48 h. A typical chromatogram of mouse urine after derivatization showing four drug-derived peaks is shown in Fig. 4. Parental sulfamic acid diester was detected as the diiodo derivative, 1,7-diiodoheptane. 7-Iodo-1-heptanol has previously been shown to be formed by derivatization of sulfamic acid 1,7-heptanemonoyl ester [3]. The amount of sulfamic acid 1,7-heptanemonoyl ester in mouse urine was 12% of the amount of sulfamic acid diester, which is consistent with its formation by breakdown over 8 h in urine [3]. The chromatographic peak occurring at 15.5 min was identified by mass spectral analysis as 3-hydroxyl-1,7-iodoheptane probably derived from sulfamic acid, 3-hydroxyl-1,7-heptanediyl ester (3-hydroxysulfamic acid diester). The electron impact mass spectrum of trimethylsilylated 3-hydroxyl-1,7-iodoheptane contained a molecular ion at m/z 425 (Fig. 6). The other fragment ions shown indicate fragmentation along the hydrocarbon backbone. The amount of the fourth drug-derived peak was not high enough to permit identification by mass spectral analysis. The percentage of excretion of the different metabolite/breakdown products in mouse urine is shown in Fig 7.

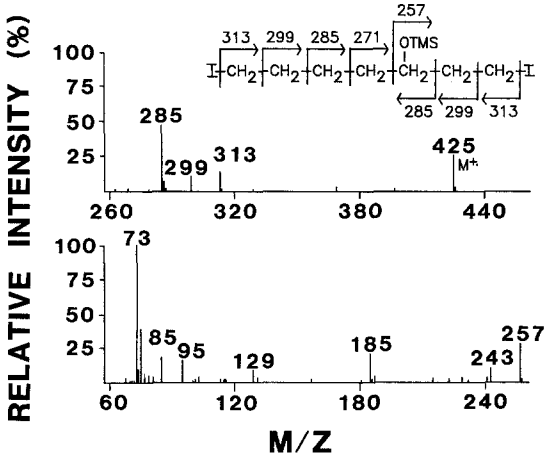

Fig. 6. Mass spectrum, 70-eV electron-impact, of the gas chromatographic peak of trimethylsilylated 3-hydroxyl-1,7-iodoheptane. The 3-hydroxyl-1,7-iodoheptane was formed by incubation of 4 *M* sodium iodide with 48-h urine from a beagle dog given 8.5 mg/kg sulfamic acid diester by i.v. bolus. Following derivatization with sodium iodide, the 3-hydroxyl-1,7-iodoheptane was converted to the trimethylsilyl ester derivative by silylation with *N,O-bis* (trimethylsilyl) trifluoroacetamide for 10 min at room temperature

In vitro metabolism. Isolated rat hepatocytes could convert sulfamic acid diester to a product giving 3-hydroxyl-1,7-iodoheptane following derivatization (Table 2). The reaction rate was linear for 180 min and was completely inhibited by preheating hepatocytes to 100° C for 3 min. The microsomal fraction also metabolized sulfamic acid diester to this product (Table 2). Preheating microsomes to 100° C for 3 min completely inhibited the product formation. The amount of sulfamic acid 1,7-heptanemonoyl ester seen in both hepatocyte and microsomal incubations was < 5% of the sulfamic acid diester, which is consistent with its formation during the assay derivatization procedure [3] and not by metabolism. No other metabolites were detected in either microsomal or hepatocytic incubations by the GC assay used.

Reaction with glutathione

The ability of sulfamic acid diester to react directly with glutathione was studied by following the disappearance of glutathione. There was no loss of glutathione over 1 h when 100 μg/ml sulfamic acid diester (0.3 m*M*) was incubated with 0.2 m*M* glutathione in buffer (pH 7.0 or 8.5) or with 100,000 *g* rat liver cytosol and 1.0 m*M* glutathione (results not shown).

Discussion

Sulfamic acid diester is eliminated at a faster rate from the plasma of mouse and dog than from whole blood. Previous studies have shown that sulfamic acid diester incubated in vitro with human whole blood exhibits concentration-dependent breakdown and sequestration by blood cells [3]. At 50 and 100 μg/ml sulfamic acid diester added to whole blood, the half-time was 6.9 h and approximately 65% of the drug was sequestered by the blood cells. At 10 μg/ml sulfamic acid diester added to whole blood, there was no detectable breakdown of the drug over 24 h and all the drug was present in the blood cells. The in vitro sequestration of sulfamic acid diester by blood cells correlates with the in vivo elimination of sulfamic acid diester from whole blood seen in the mouse and dog. In both species, the whole blood concentration of sulfamic acid diester declined to 5–10 μg/ml during the beta phase of elimination and then entered a prolonged gamma phase of elimination

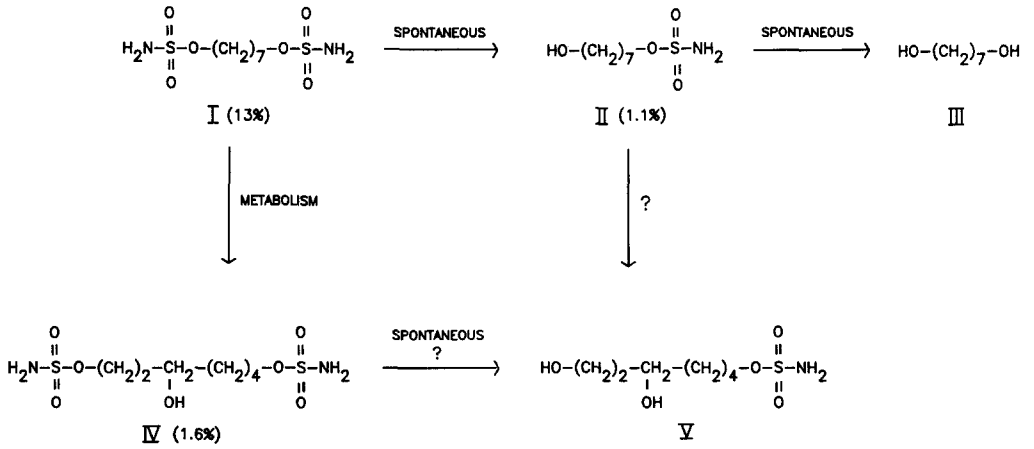

Fig. 7. Pathway for formation of metabolites and breakdown products of sulfamic acid diester. Structures are: *I*, sulfamic acid diester; *II*, sulfamic acid 1,7-heptanemonoyl ester; *III*, 1,7-heptanediol; *IV*, 3-hydroxysulfamic acid diester; *V*, sulfamic acid 3-hydroxy-1,7-hep tanemonoyl ester. Values in parentheses are the percentages excreted in mouse urine over 8 h, assuming a similar GC detector response to sulfamic acid diester (for *IV*) and to sulfamic acid 1,7-heptanemonoyl ester standard

with a half-life of 32 h in the mouse and 42 h in the dog. In addition, during i.v. infusion of sulfamic acid diester to CDF_1 mice, sulfamic acid diester was rapidly taken up by the blood cells, reaching a steady-state concentration of 9.6 μg/ml despite a slow, continued rise of plasma sulfamic acid diester.

Sulfamic acid diester could not be removed from lysed blood cells by dialysis and no sulfamic acid diester was found in the ultrafiltrate. This suggests a strong interaction between sulfamic acid diester and the blood cells. Busulfan, a structurally similar alkylating agent, has been reported to bind both reversibly and irreversibly to blood cells [4]. Depending on the degree of reversibility of the blood cell-sulfamic acid diester interaction in vivo, sequestration by the blood cells is a potential mechanism for the storage and slow release of sulfamic acid diester. In addition, sequestration of sulfamic acid diester by the blood cells might effect the disposition of the drug in situations where blood cell counts are abnormally high or low.

Sulfamic acid diester, an electrophile, might be expected to interact with thiol compounds. The ability of sulfamic acid diester to react with glutathione was studied by following the disappearance of glutathione from whole blood and from buffer containing glutathione. Sulfamic acid diester was found not to react directly with glutathione. The inclusion of rat liver cytosol, which contains glutathione S-transferases [7], did not facilitate the reaction of sulfamic acid diester with glutathione.

Table 2. Metabolism of sulfamic acid diester by rat liver

	Metabolite appearance (ng/min/mg or 10^6 cells)
Hepatocytes	
5% CO_2 : 95% air	0.7 ± 0.5
5% CO_2 : 95% air, boiled	0
Microsomal fraction	
+ NADPH	1.2 ± 0.3
+ NADPH, boiled	0

Sulfamic acid diester (100 μg/ml) was incubated with 5×10^6 hepatocytes/ml in Dulbecco's modified Eagles medium containing 10% fetal calf serum at 37°C for 360 min. Alternatively, sulfamic acid diester (100 μg/ml) was incubated with 2.5 mg microsomal protein/ml and an NADPH-generating system, in 0.1 *M* sodium phosphate buffer (pH 7.4) at 37°C for 120 min. The values are the means of three determinations in each case. The results for metabolite formation are expressed as sulfamic acid diester equivalents, assuming a similar response with electron-capture detection

It is possible that part of the sulfamic acid diester is eliminated by metabolism in vivo, since the rate of degradation of sulfamic acid diester in biological fluids [3] is slower than its in vivo elimination. Since a metabolite would have to undergo nucleophilic substitution by iodide to be detected by the GC assay we used, it is possible that some sulfamic acid diester metabolites were not detected. Only 4% of the dose of sulfamic acid diester was excreted in the urine as unchanged drug in 48 h in the dog, and 13% in 8 h in the mouse. The urine from both mouse and dog contained three additional peaks that were not present in pretreatment urine. One peak was 7-iodo-1-heptanol formed from sulfamic acid 1,7-heptanemonoyl ester. Another peak was identified by GC mass spectral analysis as 3-hydroxy-1,7-iodoheptane and was probably formed from 3-hydroxysulfamic acid diester. A small peak was consistently seen at the same place as 3-hydroxy-1,7-iodoheptane in the GC chromatograms of the plasma following sulfamic acid diester administration, suggesting that the plasma also contained 3-hydroxysulfamic acid diester. Isolated hepatocytes and the microsomal fraction in the presence of NADPH were also found to convert sulfamic acid diester to the 3-hydroxysulfamic acid diester. A third metabolite or breakdown product peak was seen in urine and plasma and may have been formed from 3-hydroxy-1,7-heptanemonoyl ester. The completely hydrolyzed form of sulfamic acid diester, 1,7-heptanediol [13], was not detected by our GC assay and might be a major product formed in vivo. A possible metabolism/breakdown pathway for sulfamic acid diester in vivo is shown in Fig. 7.

The metabolism of sulfamic acid diester does not appear to follow the same pattern as the metabolism of its analog, busulfan. The main metabolite of busulfan identified in the isolated perfused rat liver is Y-glutamyl-β-(S-tetrahydrothiophenium) alanyl-glycine (the sulfonium ion of glutathione) [10]. Three urinary metabolites that are proposed metabolic products of the sulfonium ion of glutathione have been identified in the rat [9]. The reaction of bu-

sulfan with glutathione is thought to be mediated by glutathione-S-transferase [10]. Sulfamic acid diester, on the other hand, was found not to react with glutathione in buffer, blood, or 100,000 *g* rat liver cytosol. The major hydrolysis product of sulfamic acid diester in vitro has previously been identified as sulfamic acid 1,7-heptanemonoyl ester [3, 13]. In contrast, busulfan forms the cyclic compound tetrahydrofuran upon hydrolysis [6, 8]. Differences in the reactivity of sulfamic acid diester and busulfan might reflect differences in the mechanism of their therapeutic effect, although the relationship between the metabolism of busulfan and its anticancer activity is not clearly understood at this time.

In summary, the pharmacokinetic parameters of i.v. administered sulfamic acid diester were studied in the mouse and beagle dog. The compound is relatively rapidly cleared from the plasma but is taken up by blood cells and only slowly eliminated. Metabolites and breakdown products of sulfamic acid diester have been identified in the plasma and urine, but the majority of the drug has not been accounted for. Sulfamic acid diester does not react with glutathione, in which respect it differs from its analog busulfan.

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