Cancer Chemother Pharmacol (1987) 20: 115–124

Cancer Chemotherapy and Pharmacology

Pharmacokinetics and toxicology of sparsomycin in beagle dogs*

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Summary. Sparsomycin is a cytotoxic drug exhibiting a broad spectrum of in vitro activity against murine tumors and many tumor cell lines. It also appears to be a potent stimulator of the antitumor activity of cisplatin against L1210 leukemia in vivo. However, because of its toxicity, the antitumor activity of sparsomycin on murine tumors in vivo has been disappointing. The purpose of our study was to investigate the pharmacokinetics of this drug as well as the possible mechanisms that produce sparsomycin toxicity. Tests on beagle dogs revealed that about 60% of the drug is eliminated by metabolic clearance, while 40% is eliminated by the kidneys. After a single bolus injection of 0.1 mg/kg sparsomycin without narcosis, sparsomycin was eliminated with a $t\beta_{1/2}$ of 0.6–0.7 h, the AUC being 0.32–0.38 $mg \cdot h \cdot l^{-1}$, and the volume of distribution (V_d) 0.26 l/kg. In addition to being subject to glomerular filtration, sparsomycin is probably also actively excreted and actively reabsorbed by the renal tubuli. Sparsomycin itself may inhibit its active tubular excretion, thus resulting in a decrease in the drug's renal clearance and its accumulation in the plasma. Sparsomycin appeared to be toxic primarily in the liver, disturbing its function and the synthesis of plasma proteins. Two out of five dogs developed hemorrhagic diathesis due to hypofibrinogenemia and deficiency of other blood-coagulation factors. Sparsomycin was not toxic to the bone marrow.

Introduction

Sparsomycin (NSC-059729), which was originally isolated from a bacterial broth of *Streptomyces sparsogenes* [16], is a potent inhibitor of ribosomal protein synthesis [11]. Owen et al. have reported that sparsomycin is active on human KB cells in vitro and on several rodent tumors in vivo [16]. In 1964, the drug was given a phase-I clinical trial: five patients received sparsomycin in daily increasing doses for 12–14 days. The trial was prematurely discontinued because of retinal toxicity in two patients [3]. Nowadays, this type of scheduling is no longer used because of the high risk of cumulative drug toxicity [2]. Further preclinical research was not performed until 1981. The revival of interest in this drug is justified by the recent development of a flexible procedure for its total synthesis [15] and by the development of some carefully designed new analogs [1, 10].

Synthetic sparsomycin has recently been found to be active in vitro in several murine tumors and in many human tumor cell lines [5]. The results of in vivo screening tests performed in National Cancer Institute (NCI) laboratories over the last 20 years [12] have been disappointing when compared to the data obtained by Owen et al. [16], because the NCI was only able to confirn the antitumor activity of sparsomycin (natural product) for P388 leukemia and Walker 256 carcinosarcoma [12]. However, even though lacking antitumor activity of its own, synthetic sparsomycin has been found to potentiate the activity of cisplatin on CHO cells in vitro [27] and on L1210 leukemia in vivo, inducing 67% cures in optimalized schedules [25]. This synergistic effect, which has also been reported for other protein synthesis inhibitors [6, 7], may provide an important application of sparsomycin in cancer chemotherapy.

The reason for the discrepancy between the in vitro and in vivo antitumor activity of sparsomycin probably results from the toxicity of this drug, which does not allow effective drug levels to be reached in vivo. In a daily $\times 9$ schedule, the LD_{10} od sparsomycin in mice is about 40 times smaller than the single LD_{10} dose [12], suggesting that sparsomycin accumulates in vivo.

Experimental data suggest that a reasonable therapeutic index can only be achieved by persistent drug administration [12]. During treatment with sparsomycin, the drug-sensitive proteins disappear from cells and plasma at a rate proportional to their turnover rate [24]. Thus, proteins with a rapid turnover will disappear first, and if these proteins are critically important to the integrity of the host, they should be the best indicators of drug toxicity. The proteins that are most sensitive to inhibition biosynthesis are those with a high mRNA initiation potential, e.g., ferritin [20] and albumin [21], so that toxicological studies should be focused on liver export proteins.

To achieve the maximal antitumor effect, dose-limiting toxicities should be clearly defined, and the levels of critical proteins should be monitored.

* This work was supported by a grant from the Queen Wilhelmina Foundation Fund (SNUKC-84-1) and by Pharmachemie (Haarlem, The Netherlands). Part of this work was presented at the 14th International IUCC Congress, Budapest, Hungary, 21–27 August, 1986

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The aim of the present investigation was to analyze the pharmacokinetics of sparsomycin together with its effects on some liver-derived proteins in order to determine the possible mechanisms of sparsomycin toxicity. The results should provide guidelines for the development of better analogs of this drug.

Materials and methods

Animals. Eleven male beagle dogs (Central Animal Laboratory, University of Nijmegen) weighing between 8.8 and 14.0 kg were used. The dogs were fed with standard granulated dog food (Hope Farms, The Netherlands) and had free access to tap water. During the experiments, the animals were housed in wire-bottomed metabolic cages that enabled urine collection.

Drugs. Sparsomycin was synthesized as previously described [15] and was aquired in a freeze-dried form. The injections were prepared aseptically in the hospital pharmacy (St. Radboud Hospital). The drug was dissolved in 0.9% NaCl and kept in dark flasks at 4° C for not longer than 24 h.

Dose. In a pilot study involving 2 dogs, we had previously determined that nine daily administrations of 0.1 mg/kg sparsomycin was the maximal dose tolerated by the animals. A dose of 0.1 mg/kg (2 mg/m^2) daily is close to the maximal dose of 0.05 mg/kg (1.85 mg/m^2) used in humans [3]. A 1.4-mg/kg dose was considered to be the maximal single dose that could be tolerated. At the time of this experiment, we were not aware of relevant data published by the NCI [13]. The doses used for continuous infusions were calculated from the single-dose experiments, so that steady-state levels of 50 and 100 ng/ml could be attained. The treatment protocols are listed in Table 1.

Sparsomycin analysis. Sparsomycin concentrations in plasma and urine were determined using high-performance liquid chromatography (HPLC) as previously described [23] using a semi-automated HPLC system (Kratos Analytical, USA). For urine analysis, the method was slightly modified; instead of using methanol as the eluent, 10% acetonitrile was applied. The detection limits were 10 ng/ml for plasma and 50–80 ng/ml for urine.

Pharmacokinetic and toxicological studies. The dogs were fasted for 12 h before each experiment. Most of the pharmacokinetic studies were performed with the dogs under pentobarbital anesthesia (initial dose, 30 mg/kg) and subjected to intratracheal intubation. During each experiment (lasting 5–7 h), the animals received intravenous fluids (0.9% NaCl and 2% mannitol) at a rate necessary to produce the required urine flow (for protocol details, see Tables 1 and 2). Blood samples were taken via a central venous catheter, immediately centrifuged, and frozen at −20° C. Urine (7–10 ml) was collected through a urinary catheter. At the end of each collection period, the bladder was emptied by gentle massage. All urine analyses for the determination of sparsomycin levels, creatinine concentrations, and urinary pH were performed in duplicate. Urine samples were kept at 20° C until analysis. After the dogs had recovered from anesthesia, they were returned to metabolic cages, where urine collection and blood sampling were continued for 24 h. On days 2–8 (expt. 1a; dog 632), the sparsomycin concentration in plasma was determined in blood samples taken 5 min before, 5 min after, and 60 min after the drug injection. On days 1 and 9, the already described single-dose procedure was repeated. Another dog received a single dose of 1.4 mg/kg sparsomycin (expt. 7; dog 751), and the drug concentration was determined in bile samples collected through a catheter preoperatively inserted in the choledochal duct. Two dogs received continuous infusions of sparsomycin for 3.5 and 4 h: 1 received 0.043 mg/kg per hour (expt. 8; dog 868), and the other was given 0.025 mg/kg per hour (expt. 9; dog 785).

All pharmacokinteic parameters were calculated from plasma and urine concentrations according to well-known procedures [22].

Table 1. Treatment protocol

Expt./ dog no.	Dose (mg/kg)	Schedule	Anesthesia on day 1	Anesthesia on day 9	Additional hydration	Follow up (days)
1/632	0.1	Q01Dx09	−	−	−	60
3/890	0.1	Q01Dx09	+	+	−	60
4/892	0.1	Q01Dx09	+	+	+	4[a]
5/063	0.1	Q01Dx09	+	−	+	60
6/008	0.1	Q01Dx09	+	−	−	4[a]
Single doses						
2/350	0.1	Q01Dx01	−			5
7/751	1.4	Q01Dx01	+			0[a]
8/868	0.043 mg/kg/h	Cont.infusion (3.5 h)	+			5
9/785	0.025 mg/kg/h	Cont.infusion (4.0 h)	+			5
10/160	0.1	Q01DX01	−			5
11/172	0.1	Q01DX01	−			5
12/160[b]	0.5	Q01DX01	−			5
13/172[b]	0.5	Q01DX01	−			5

[a] The follow-up was shortened due to death

[b] The experiment was repeated on the same dogs 3 months after the experiment whose results are shown here

Toxicological study. Dogs 632, 890, 892, 063, and 008 were subjected to toxicological study following nine daily doses of 0.1 mg/kg sparsomycin. Dogs 892 and 063 additionally received daily hydration (0.5 l glucose-saline as a hypodermic clysma) in order to minimize the effects of dehydration. Blood and urine samples were analyzed for changes in biochemical and hematological parameters. Additonally, four single-dose experiments were performed (expts. 10–13; dogs 160 and 172) without anesthesia, and a pharmacokinetic study was performed to evaluate exclusively the dynamics of factor-VII changes. Factor-VII levels were determined using factor-VII-deficient human plasma and dog brain thromboplastine [18]. The 100% value (±SD) was derived from the results obtained for 12 healthy beagles not involved in this study. Thrombotests were performed according to the method of Owren [17]. Student's *t*-test was applied to assess the significance of the differences between day-1 and day-9 values; the accepted level of significance was $P < 0.05$.

Eye toxicity. In each of the dogs that received a 9-day course, fundoscopy was performed three times, i.e., on the 1st, 9th, and 16th days of the experiment. As dog 892 was killed after the experiment and dog 008 died on day 13, their retinas were subjected to histological evaluation.

Results

The pharmacokinetic results are summarized in Table 2.

Bolus injection of 0.1 mg/kg sparsomycin without anesthesia

Sparsomycin that had been administered to the 2 dogs that were not under pentobarbital anesthesia (expts. 1 and 2) was rapidly eliminated from the circulation as shown by the following values: $t\beta_{1/2}$, 0.60 and 0.68 h; AUC, 0.32 and 0.38 $mg \cdot h \cdot l^{-1}$; and volume of distribution (V_d), 0.26 and 0.26 l/kg. The drug was eliminated mainly by nonrenal clearance (CL_{NRSm}), with 40% of the administered dose being consistently recovered in the urine after 24 h.

Bolus injections of 0.1 mg/kg under anesthesia

The dogs that received bolus injections while under pentobarbital anesthesia (expts. 3 and 4) eliminated sparsomycin less rapidly having $t\beta_{1/2}$ values of 0.7 and 0.76 h. In these experiments, the AUCs values increased to 0.99 and 0.78 $mg \cdot h \cdot l^{-1}$ mainly because of a slow γ-phase with $t\gamma_{1/2}$ values of 7.9 and 9.4 h. The results of expt. 3 are shown in Fig. 1.

The increase in the AUC values was mainly due to reduced CL_{NRSm}, as the renal clearance (CL_{RSm}) remained the same, i.e., about 20 ml/min. As a consequence of the

Table 2. Pharmacokinetic data for sparsomycin administered as an i.v. bolus or by continuous infusion

Experiment	Expt.	Dog no.	Body weight (kg)	Mean urine flow ± SD (ml/min)	$t\beta_{1/2}$ (h)	AUC ($mg \cdot h \cdot l^{-1}$)	V_d (l/kg)	CL_{CR} (ml/min)	CL_{RSm} (ml/min)	Percentage of the dose excreted in urine	CL_{Sm} (ml/min)
Bolus injection without anesthesia (0.1 mg/kg) (day 1)	1	632	12.5	0.6 ± 0.5	0.60	0.32	0.26	–	24.6	40.0	62.0
	2	350	13.1	0.7 ± 0.6	0.68	0.38	0.26	–	23.8	39.5	60.2
Bolus injection under anesthesia (0.1 mg/kg) (day 1)	3	890	13.0	0.2 ± 0.05	0.70	0.99	0.26	40.4 ± 17	18.4	84.0	22.0
	4	892	12.5	0.2 ± 0.05	0.76	0.78	0.57	34.3 ± 3	21.6	81.0	26.7
Bolus injection under anesthesia; (0.1 mg/kg) high urine flow (day 1)	5	063	11.8	1.7 ± 0.08	1.17	0.40	0.42	42.3 ± 7	34.4	70.0	49.2
	6	008	8.8	1.9 ± 0.08	0.83	0.34	0.36	35.7 ± 11	29.3	68.0	43.1
Prolonged drug admin.	1b[a]	632	10.7	0.2 ± 0.5	0.76	1.87	0.26	–	3.6	38.0	9.5
0.1 mg/kg; Q01Dx9 (day 9)	3a	890	11.9	0.2 ± 0.04	1.17	1.74	0.27	35.9 ± 16	10.5	100.0	10.5
	4a[b]	892	11.7	0.3 ± 0.08	0.64	0.74	0.32	21.1 ± 9	21.0	80.0	26.4
Bolus injection under anesthesia (1.4 mg/kg) (day 1)	7	751	11.2	1.0 ± 0.09	1.0	10.80	0.25	–	–	55.0	25.6
Continous infusion, under anesthesia; high urine flow:											
0.043 mg/kg/h	8	868	14.0	1.3 ± 0.46	0.60	0.72	–	47.9 ± 9	30.3	62.0	49.0
0.025 mg/kg/h	9	785	13.5	1.0 ± 0.32	0.54	0.35	–	45.3 ± 7	45.0	73.0	61.7

[a] Experiment 1b was performed without anesthesia
[b] Additional hydration on days 2–8

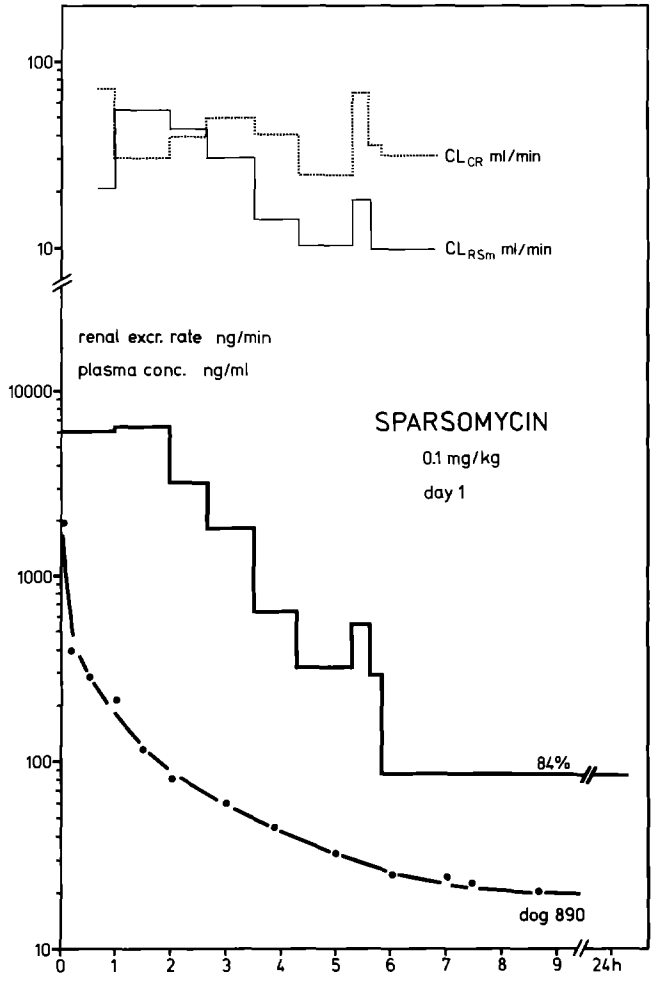

Fig. 1. Experiment 3: plasma concentration time curve (●—●) and renal excretion rate time profile (———) of sparsomycin administered as a bolus i.v. dose (0.1 mg/kg) to dog 890 under anesthesia on day 1 of the experiment. The percentage of the dose recovered in the urine after 24 h is indicated on the last renal excretion rate bar. Changes in the CL_{CR} (.....) and CL_{RSm} (———) are shown in the *upper part* of the figure. $t\beta_{½}$, 0.7 h; $t\gamma_{½}$, 7.9 h

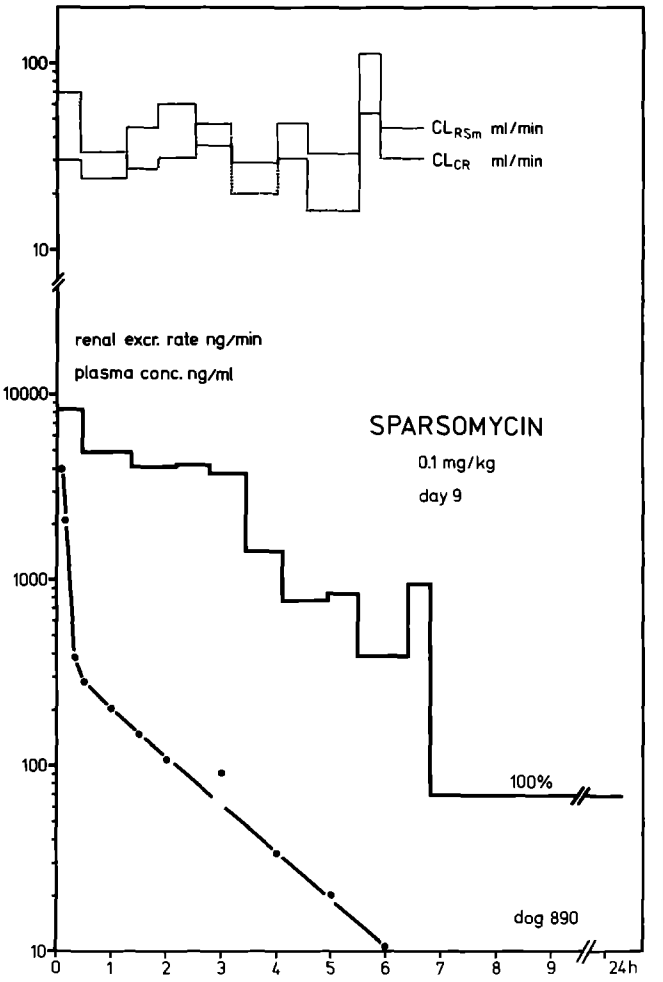

Fig. 2. Experiment 3a: sparsomycin administered as a bolus i.v. dose (0.1 mg/kg) to dog 890 under anesthesia on day 9 (the last of nine daily doses). $t\beta_{½}$, 1.17 h; the γ-phase was absent. For further details, see the legend to Fig. 1

decreased CL_{NRSm}, most of the drug was excreted in the urine (84% and 80% of the administered dose). The volume of distribution (V_d) values in these two experiments were 0.26 and 0.57 l/kg. These results were obtained with a relatively low urine flow of 0.2 ml/min.

Influence of high urine flow on renal drug clearance

An increase in the urine flow to 1.7 and 1.9 ml/min in expts. 5 and 6, respectively, resulted not only in increases in the CL_{RSm} to 34.4 and 29.3 ml/min, respectively, but also an increase of the CL_{NRSm} to about 14 ml/min (see Fig. 3). The proportions of the drug recovered in the urine were 70% and 68%, respectively. The elimination values, $t\beta_{1/2}$, did not drop in these experiments but increased to 1.17 and 0.83 h, respectively. An increase in the urine flow resulted in the disappearance of the γ-phase and low AUC values of 0.4 and 0.34 $mg \cdot h \cdot l^{-1}$. Despite the high urine flow, the CL_{RSm} decreased significantly during the 4th h of the experiment but thereafter increased. Under vigorous hydration, the V_d values in these experiments increased to 0.42 and 0.36 l/kg, respectively (a 35% increase).

Elimination profile of sparsomycin after daily administration (× 9)

Dogs 632, 890, and 892 were given nine daily i.v. sparsomycin doses of 0.1 mg/kg. In dog 632, a pharmacokinetic study without anesthesia was performed on days 1 (expt. 1) and 9 (expt. 1a), and on days 2–8, additional blood samples were taken 5 min before the injection, and 5 and 60 min after the injection.

Dog 632 became ill during the treatment course. Vomiting and anorexia resulted in weight loss (−1.8 kg) and dehydration. On day 9 the dog produced only 110 ml concentrated urine per day (normal level, 500–750 ml/day). From days 1 to 8, the daily measurements of plasma drug levels (expt. 1a) revealed that the peak levels (5 min after injection) remained the same, i.e., 529 ± 53 ng/ml. This was also the case for the drug levels 60 min after the injection, i.e., 94 ± 16 ng/ml. Residual drug levels at 5 min before the injection were detectable only on days 5 (14 ng/ml) and 7 (10.5 ng/ml). On day 9 (expt. 1b), the drug-elimination profile changed significantly: the residual drug level increased to 75 ng/ml, there being a peak value of 605 ng/ml and a 60-min value of 210 ng/ml. The β-phase with a $t\beta_{1/2}$ of 0.76 h was followed by a γ-phase with a $t\gamma_{1/2}$

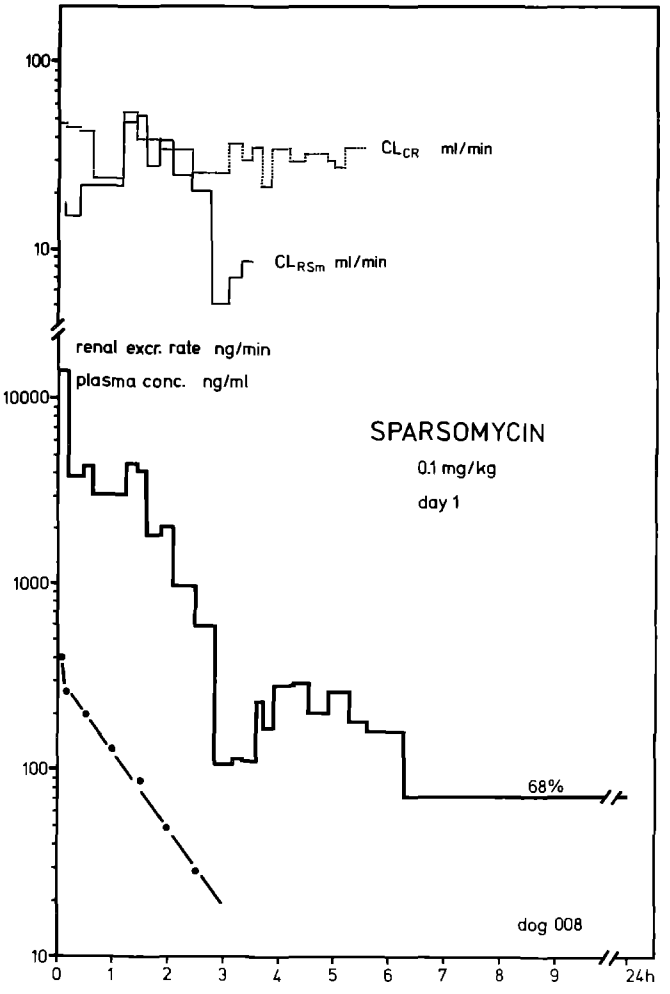

Fig. 3. Experiment 6: sparsomycin administered as a bolus i.v. dose (0.1 mg/kg) to dog 008 under anesthesia with vigourous hydration on day 1 of the experiment. $t\beta_{1/2}$, 0.83 h; the γ-phase was absent. For further details, see the legend to Fig. 1

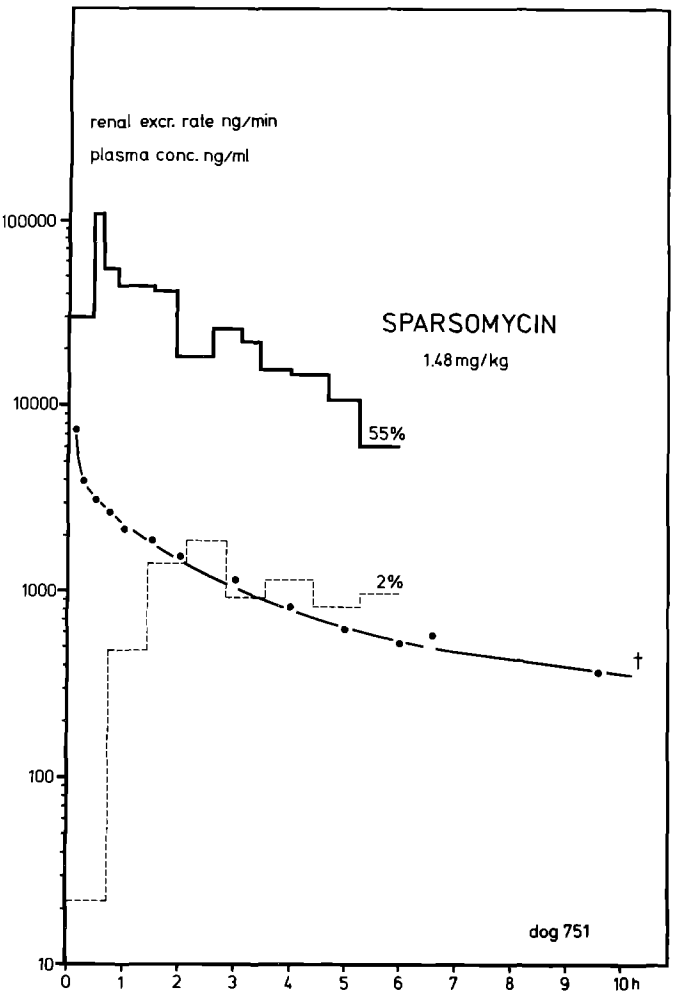

Fig. 4. Experiment 7: sparsomycin administered as a bolus i.v. dose (1.48 mg/kg) to dog 751 under anesthesia. The percentage of the dose recovered in the urine after 6 h is indicated on the last renal excretion rate bar. Sparsomycin concentrations in the bile (———) and the percentage of the dose recovered in the bile are indicated. $t\beta_{1/2}$, 1.0 h; $t\gamma_{1/2}$, 6.7 h

of 20 h. The AUC increased to 1.87 $mg \cdot h \cdot l^{-1}$. Dog 632 recovered promptly within 10 days of the last sparsomycin injection; it started to gain weight and had normal diuresis.

Dogs 890 and 892 (expts. 3 and 4) were subjected to a procedure similar to that used for dog 632, except that the pharmacokinetic studies on days 1 and 9 were performed under anesthesia. Dog 892 received additional hydration throughout the experiment (days 2–8, 0.5 l saline-glucose as a daily hypodermoclysis). The elimination profile of sparsomycin in expts. 3 and 4 on day 1 have been described above. On day 9 (expts. 3a and 4a), the drug-elimination profiles were different. Dog 890, which did not receive additional hydration, showed a markedly increased $t\beta_{1/2}$ value of 1.17 and an AUC value of 1.74 $mg \cdot h \cdot l^{-1}$ 9 (expt. 3a; Fig. 2). Apparently, the slow γ-phase had disappeared, and 100% of the administered dose was recovered in the urine. The total body clearance (CL_{Sm}) decreased to 10.5 ml/min and was equal to the CL_{RSm}. Dog 892, which was hydrated on days 2–8, showed an unchanged drug-elimination profile in expt. 4a, i.e., a $t\beta_{1/2}$ value of 0.64 h, an AUC of 0.74 $mg \cdot h \cdot l^{-1}$, a persisting γ-phase. The CL_{Sm} remained as high as on day 1, and 80% of the initial dose was recovered in the urine.

The pentobarbital sleeping times after an initial dose of 30 mg/kg pentobarbital were recorded for both dogs during the first and second anesthesia. On day 1, dogs 890 and 892 woke up after 1.1 and 1.4 h, respectively, and an additional pentobarbital dose was necessary to keep them asleep. On day 9, the times were 23 and 30 h, respectively, for the same pentobarbital dose. When dog 892 woke up on day 10, it was still stick and hypothermic, and it remained so until day 13, when it was killed for humanitarian reasons. Death was not directly due to sparsomycin toxicity, but rather was attributable to an unexpected delay in pentobarbital metabolism.

High sparsomycin dose: 1.48 mg/kg as an i.v. bolus

In expt. 7, sparsomycin at a dose of 1.48 mg/kg was injected under anesthesia as an i.v. bolus (Fig. 4). The $t\beta_{1/2}$ was 1.0 h, and the AUC was 10.8 $mg \cdot h \cdot l^{-1}$. The dog died after 10 h, and the amount of the drug recovered in the urine up to the time of death was 55% of the administered dose. Simultaneously measured sparsomycin concentrations in the bile revealed that only 2% of the administered dose had

been eliminated in an unchanged form by this route. Autopsy failed to reveal the cause of death.

Continuous infusion of sparsomycin

Sparsomycin given as a continuous i.v. infusion (0.043 mg/kg per hour; expt. 8) resulted in steady-state plasma concentrations during the first 100 min of the experiment. At the same time, the CL_{RSm} increased from 20.0 to 45.0 ml/min, thereby approximating the creatine clearance, CL_{CR} (mean, 47.9 ± 9.0 ml/min). The CL_{RSm} remained at a constant level of 40–45 ml/min for only 60 min and then started to decline; this resulted in increased sparsomycin levels in the plasma. After the discontinuation of sparsomycin infusion, the drug was eliminated with a $t\beta_{1/2}$ of 0.60 h. When the sparsomycin plasma concentration dropped to about 60 ng/ml, the CL_{RSm} increased once again to 50 ml/min but had decreased by the end of experiment. The CL_{Sm} observed during this experiment was 49.0 ml/min, while 62% of the sparsomycin dose was recovered in the urine.

The urine pH dropped from 7.7 at the beginning of the experiment to 6.5 at the end of drug infusion, and had returned to a value of 8.0 at the end of experiment. The urine flow was constant during the drug infusion, but increased from 1.0 to 2.5 ml/min immediately after discontinuation of the infusion.

The same experiment was repeated using one-half of the previous dose (0.025 mg/kg per hour; expt. 9); the results are shown in Fig. 5. After an initial rise in the CL_{RSm} to 149 ml/min, the clearance deteriorated to values lower than the mean CL_{CR} (45.3 ± 7.0 ml/min). After an initial steady state, the plasma levels of sparsomycin began to increase during the 2nd h of the experiment. Again, after discontinuing drug infusion, sparsomycin was eliminated with a $t\beta_{1/2}$ of 0.54 h. The CL_{RSm} was restored to a value of 50 ml/min when the plasma levels dropped below 60 ng/ml. The CL_{Sm} was 61.7 ml/min, and 73% of the sparsomycin dose was recovered in urine after 24 h. To control variations in the urine flow, a volumetric infusion pump with a constant fluid-infusion rate of 3.0 ml/min was used. After an initial equilibration time of 30 min, the urine flow remained constant at 1.1 ± 0.26 ml/min until sparsomycin infusion was terminated, at which time the urine flow increased to 2.3 ml/min. The changes in urine pH were similar to those observed in expt. 8.

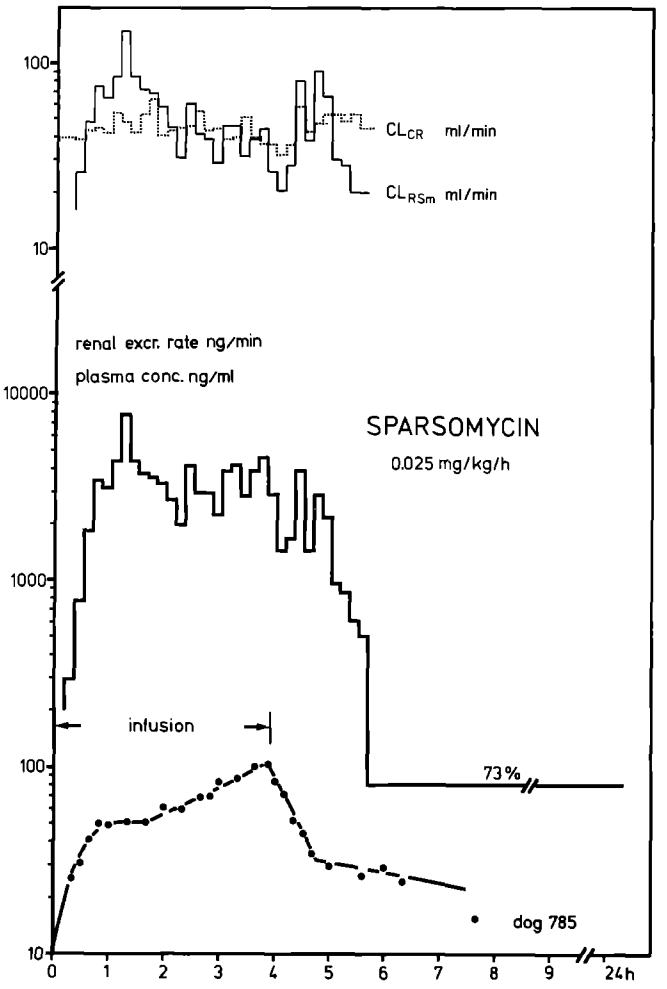

Fig. 5. Experiment 9: sparsomycin administered as a continuous infusion (0.025 mg/kg per hour) to dog 785 under anesthesia with vigourous hydration. $t\beta_{1/2}$, 0.54 h; $t\gamma_{1/2}$, about 8 h. For further details, see the legend to Fig. 1

Toxicology

As a result of daily treatment with 0.1 mg/kg sparsomycin for 9 days, the 5 animals (dogs 632, 890, 892, 063, and 008) lost body weight (mean loss, −1.8 kg; not significant: see Table 3) and became anorexic. One dog (008) died because of dehydration, uremia, and hemorrhagic diathesis on day 13, while another (892) was killed on day 13 because it did not recover from the pentobarbital anesthesia given on day 9. The dogs drank water normally but vomited immediately after. This phenomenon was most apparent about 1 h after drug injection and resulted in significant hemoconcentration and a decrease in urine production (dogs 632, 890, and 008). The dogs that were additionally hydrated (063 and 892) exhibited less hemoconcentration and normal diuresis (about 500 ml/day). Decreased renal creatinine clearance and weight loss were seen in all 5 dogs. During therapy, the urinary sodium excretion was significantly reduced to values of <10 mEq/l per day, while the urine osmolality remained relatively high. In the 2 dogs that received additional fluid and sodium, the urinary sodium excretion level increased to >20 mEq/l per day. Despite the considerable decrease in the creatinine clearance ($P = 0.02$), the plasma creatinine values remained unchanged, and plasma urea levels decreased in all but 1 dog ($P = 0.09$). Dog 008 developed overt renal insufficiency and died on day 13. In addition to generalized hemorrhagic diathesis, a regenerating proximal-tubular necrosis was found at autopsy.

Small amounts of glucose were found in one urine sample (dog 632) after 9 days of treatment. The loss of urine protein never exceeded 2 g/day (61 collections).

In all dogs, plasma electrolytes remained at normal levels during and after sparsomycin therapy. However, the plasma Ca^{2+} level dropped significantly ($P = 0.02$), probably because of the decrease in plasma albumin.

Total plasma protein (TP) decreased from 60 h to 55 g/l but this decrease was masked by hemoconcentration. The plasma albumin level decreased from 29.7 ± 1.8 to 21.2 ± 2.7 g/l in 8 days ($P = 0.003$; Table 3). The mean disappearance half-life time ($t_{1/2}$) was 20.5 days. After the

discontinuation of sparsomycin, plasma albumin returned to the pretreatment values within 10 days. The levels of plasma globulins (TP-albumin) did not change during the experiments.

The plasma fibrinogen level decreased dramatically from 1276±98 to 291±265 ($P = 0.003$). The disappearance $t_{1/2}$ was 3.5 days. The 2 dogs with the lowest plasma fibrinogen level on day 9 (dog 892, 160 ml/l; dog 008, <100 mg/l) showed a considerable tendency towards bleeding in the last 2 days of sparsomycin treatment. In these 2 dogs, gingival bleeding and bloody diarrhea were observed. Both dogs died or were killed on day 13. Prior to death, both dogs had supranormal plasma fibrinogen levels.

The level of plasma factor VII decreased rapidly after the first sparsomycin dose. The mean disappearance $t_{1/2}$ was 5.6±1.5 h (see Fig. 6). The effect of the five-times-higher sparsomycin dose was only slightly stronger, the $t_{1/2}$ being 4.2 h. Six to ten hours after drug injection, a plateau of 31±8% of the normal value was observed. At 24 h following a single sparsomycin dose of 0.1 mg/kg, the level of plasma factor VII started to recover (see Fig. 7). The recovery was slower than the disappearance and only reached the 100% level 50 h after the injection. Later on, a small overshoot of plasma factor-VII levels was observed. The recovery after a 0.5-mg/kg dose was delayed for an

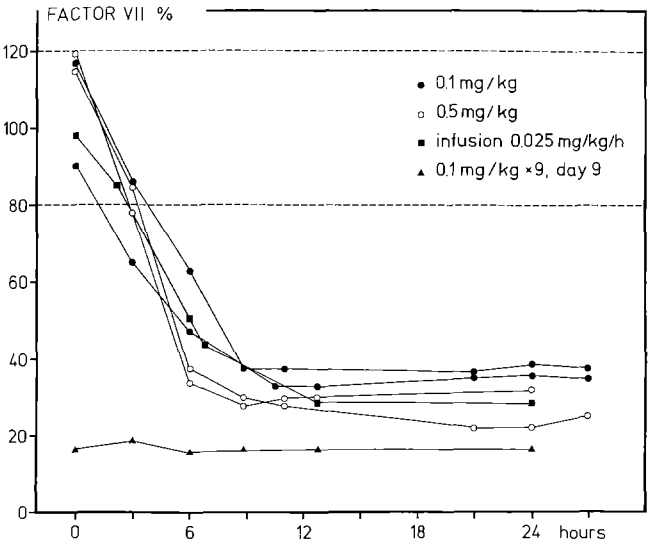

Fig. 6. Changes in plasma factor-VII levels after sparsomycin administration; the sparsomycin doses and schedules are indicated. The SD from the 100% level (mean for 12 normal dogs) is indicated by *dashed lines*

Table 3. Changes in toxicological parameters measured before treatment (day 1) and on day 9 of treatment with sparsomycin (0.1 mg/kg per day)[a]

Parameter	Units	Day 1	Day 9	P	Comments
Body weight	kg	11.8 ± 1.7	10.0 ± 1.8	ns	Recovery within 10 days
Hemoglobin	mmol/l	9.6 ± 1.1	10.9 ± 0.1	ns	Recovery within 2–3 days
Hematocrit	l/l	0.46 ± 0.04	0.51 ± 0.05	ns	
WBC	$\times 10^9$/l	10.2 ± 1.7	16.9 ± 12	ns	Two dogs died;
PMN leukocytes	$\times 10^9$/l	6.8 ± 1.0	12.7 ± 10	ns	during the experiment, their
Lymphocytes	$\times 10^9$/l	2.7 ± 0.9	2.0 ± 1.0	ns	WBC counts rese to reach
Thrombocytes	$\times 10^9$/l	159.0 ± 59	110.0 ± 29	ns	66.1 and 49.2 just prior to death
Reticulocytes	%0	9.0 ± 5	0.4 ± 0.8	0.02	Recovery within 3–5 days
Total protein	g/l	60.4 ± 6.9	55.0 ± 11	ns	Recovery within 10 days
Albumin	g/l	29.7 ± 1.8	21.2 ± 3	0.003	
Fibrinogen	mg/l	1276.0 ± 98	291.0 ±265	0.003	Recovery within 2 days
Thrombotest	%	>100	9 ± 4	0.003	Recovery within 3 days
SGOT	IU/l	31.0 ± 9.7	55.8 ± 28	ns	All liver enzymes returned
SGPT	IU/l	52.4 ± 13.5	65.4 ± 24	ns	to normal levels within 10 days
LDH	IU/l	352.6 ±181	289.0 ±151	ns	One dog had overt hepatitis
Alkaline phosphatase	IU/l	38.6 ± 10.7	64.6 ± 24	ns	
γ-GTP	IU/l	8.0 ± 4.2	17.6 ± 9.4	ns	
Na^+	mmol/l	147.0 ± 2.6	143.0 ± 8	ns	
K^+	mmol/l	3.3 ± 0.4	3.3 ± 0.7	ns	
Ca^{2+}	mmol/l	2.4 ± 0.2	2.2 ± 0.1	0.02	Recovery within 10 days
Glucose	mmol/l	5.2 ± 0.6	4.4 ± 1.1	ns	
Urea	mmol/l	6.5 ± 1.9	3.1 ± 0.7	ns	Recovery within 2–4 days
Creatinine	μmol/l	66.6 ± 18	65.0 ± 10.3	ns	
Creatinine clearance	ml/min	34.4 ± 7	17.4 ± 9.2	0.03	Recovery within 10 days. One dog did not recover until 21 days after sparsomycin administration. One dog died because of uremia

ns, not significant

WBC, leukocytes; PMN leukocytes, granulocytes (PMN-polymorphonuclear leukocytes)

[a] Mean ± SD for 5 dogs

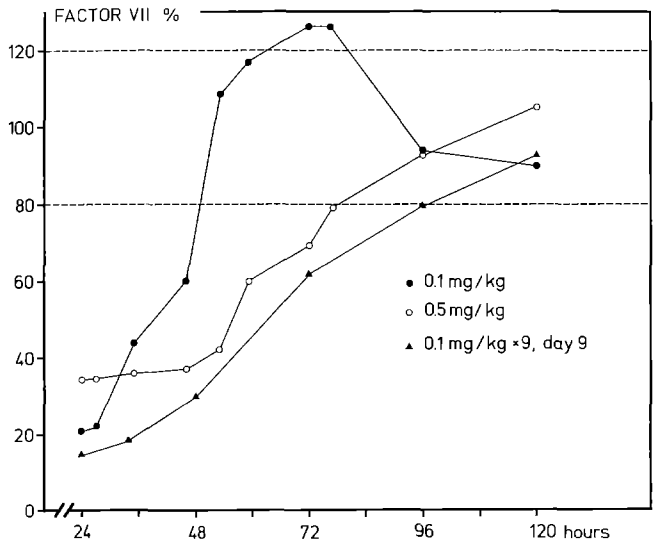

Fig. 7. Recovery of plasma factor-VII levels after the final sparsomycin administration

additional 24 h and was slower than in the case of the lower dose: the 100% value was not reached until 108 h after drug injection. When sparsomycin was administered for 9 days (dog 063), plasma factor-VII levels remained at a plateau for 9 days. After the discontinuation of sparsomycin therapy, the plasma factor-VII recovery approximately paralleled that observed after a single 0.5-mg/kg dose.

Dog 785 appeared to be deficient in factor VII, having 'plateau' levels of this protein even before drug administration. The administration of sparsomycin did not change this plateau level.

The thrombotest findings showed control levels (> 100%) for 2 days and later started to decline rapidly. On day 9, a mean value of 9%±4% had been reached (P = 0.002). The 2 dogs with hemorrhagic diathesis had values of 5% and 6%.

The liver enzymes serum glutamine-oxolate transferase (SGOT), serum glutamine-pyruvate transferase (SGPT), alkaline phosphatase, and γ-glutamyl transpeptidase (γ-GTP) showed elevated levels during and after treatment with sparsomycin. There was no apparent difference between anesthesized and nonanesthesized dogs. However, the significance of these changes was difficult to evaluate because of considerable scatter. One of the dogs (063) developed overt toxic hepatitis after the discontinuation of treatment. The derangements in liver enzyme levels in this dog were maximal on day 15, when SGOT increased to 280 IU/l, SGPT to 1,250 IU/l, and γ-GTP to 60 IU/l. Lactate dehydrogenase (LDH) and alkaline phosphatase were not excessively elevated. The derangements disappeared spontaneously, and all liver enzymes in all dogs were at normal levels on day 21 (12 days after last sparsomycin injection).

Eye toxicity

There were no changes in retinal appearance when eyes were checked by performing routine fundoscopy. At autopsy (dogs 892 and 008), no morphological changes in the retina were seen.

Discussion

In our experiments, we treated dogs with a sparsomycin dose similar to the maximal dose used in phase-I trials in humans [3].

Dogs under anesthesia had much lower CL_{NRSm} values than dogs without anesthesia. This might have been due to the specific (enzyme saturation) or nonspecific (decrease in liver blood flow) effects of pentobarbital. The increase in CL_{NRSm} values in expts. 5 and 6 when vigorous hydration was performed, the expansion of the V_d, and the relative decrease in the amount of the drug recovered in urine all point to the second possibility.

In dogs with a low urine flow, a slow γ-phase ($t\gamma_{1/2}$, about 8.0 h) was seen. The CL_{RSm} was very low at this time, suggesting that all of the filtered sparsomycin was (actively?) reabsorbed by the renal tubuli. This phenomenon might be an important factor in producing increased drug toxicity. However, the γ-phase was not seen in the 2 nonanesthesized dogs. An increase in the urinary flow (expts. 5 and 6) may result in a decrease in the tubular resorption of the drug and, therefore, a disappearance of the γ-phase. On the other hand, dehydration and low urine flows probably result in an increase in urine sparsomycin concentration along with a decrease in the level of the enzyme responsible for drug reabsorption. In such a case, all of the filtered sparsomycin is excreted in urine (expt. 3a). Additional hydration and normal diuresis would prevent this effect (expt. 4a).

Prolongation of the pentobarbital sleeping time indicates that the activity of cytochrome P450 is low. Thus sparsomycin might influence the liver metabolism of other drugs.

Drug accumulation did not exclusively occur in anesthesized dogs. In dog 632 (expt. 1a), the blood sparsomycin levels were not elevated until the last day of treatment. This observation suggests that, in spite of decreased sparsomycin renal excretion, the liver metabolism or binding capacity was still active or undersaturated until the 9th day of treatment. From the rapid deterioration of the general condition of this dog, it was clear that the observed plasma drug accumulation would have resulted in lethal toxicity if the experiment had continued.

Continuous sparsomycin infusion provided other interesting data, particularly that the CL_{RSm} could be increased far above the mean CL_{CR}. This shows that sparsomycin is also actively excreted by renal tubuli. Active excretion can be 'auto-inhibited' by sparsomycin, so that the drug clearance drops. This results in acute drug accumulation in the plasma. This phenomenon cannot be compensated for by vigorous hydration and increased urine flow. Note that a similar phenomenon was also observed for i.v. bolus injections in expts. 5 and 6 (Fig. 3): the CL_{RSm} dropped at the end of experiment but climbed again when the sparsomycin plasma levels were no longer measurable. It is difficult to propose a reasonable explanation for this phenomenon, as up to now, only a few protein-synthesis inhibitors have been studied clinically. However, on the basis of our observations, it can be speculated that sparsomycin either inhibits the synthesis of a short-lived transport enzyme in the renal tubuli or that it saturates the capacity of such an enzyme. Another possibility is that pentobarbital interacts with sparsomycin at the level of the renal tubuli.

From the results of our continuous-infusion studies, it can be anticipated that the use of a higher sparsomycin dose would result in nonlinear kinetics with delayed elimination. This did occur, and proved fatal in expt. 7 (see Fig. 4).

The increase in the urine flow and CL_{RSm} after the discontinuation of drug infusion suggests the existence of an unknown vasodepressive activity produced by sparsomycin.

In the view of dose scheduling in the phase-I study performed in 1964 [3], it might be argued that the eye toxicity observed in these trials might also have been due to drug accumulation. This supposition is based on the following considerations. The human blood-retina barrier is usually impermeable to water-soluble drugs [4]. Moreover, extensive studies on healthy animals have also failed to confirm the retinal toxicity seen in humans [14]. Our present findings of an absence of eye toxicity are in agreement with those of previous studies. The permeability of the blood-retinal barrier, however, can increase in different diseases [4], and it is also conceivable that it could be changed under different debilitating conditions, e.g., after eye irradiation and/or cancer cachexia. Both patients who suffered from retinal toxicity had a low initial body weight (50 kg). Thus, it is possible that increased drug plasma levels in combination with increased blood-retinal barrier permeability could be the reasons for the eye toxicity seen in humans.

Our study of beagle dogs was extended in order to answer some questions concerning the toxicology of sparsomycin. These studies revealed the influence of sparsomycin on liver function. Several of the proteins studied showed a decrease in their levels that was proportional to their normal turnover time.

Plasma factor VII, which is known to have a short biological half-life of 4–6 h in humans, showed rapid changes after sparsomycin administration to dogs. After an initial decay to a 30% level, a plateau was observed in all cases. This plateau, however, was an artefact of the method applied, as human plasma free of factor VII was used. This phenomenon has been described and quantified by Poller et al. [18] in factor-VII-deficient dogs. Our results, therefore, indicate the total disappearance of factor VII from the plasma of dogs treated with sparsomycin. Using our method, however, we were able to determine the length of time between the inhibition and restoration of plasma factor-VII synthesis, this being one aim of the study. As in untreated factor-VII-deficient dogs [18], the absence of this coagulation factor did not increase the risk of spontaneous hemorrhage.

The disappearance of factor VII from plasma was not related to the sparsomycin dose, as 0.5 mg/kg had nearly the same effect on this protein as 0.1 mg/kg dose and continuous infusion. This suggests that, over a broad dosage range, the toxicity of sparsomycin may be dependent not on the dosage but on the lenght of the therapy and the disappearance of critical proteins ('horizontal' limit). High sparsomycin concentrations may inhibit a wider range of cellular biosynthesis and a wider range of potentially critical proteins (respiratory enzymes?) and thereby cause death ('vertical' limit).

Factor-VII assays, although useless for predicting the risk of hemorrhage, might provide sensitive dynamic parameters for showing the persistence of protein-synthesis inhibition in liver cells. Theoretically, in our experiments, each daily dose was administered just before the recovery of factor-VII plasma levels. The drug doses should have been more spread out during the second part of experiment, as sparsomycin appeared to accumulate in plasma. It is also of interest to note that the recovery of factor-VII levels was several times slower than its disappearance, which suggests either low synthetic priority or increased consumption of factor VII.

The decrease in plasma fibrinogen levels was well correlated with drug toxicity, and the duration of therapy was clearly limited by this effect. The disappearance $t_{1/2}$ value of 3.5 days was very similar to that of human fibrinogen, i.e., 3–4 days [9]. After the discontinuation of therapy, fibrinogen synthesis recovered promptly, but the exact time of recovery was not estimated in our study. Plasma fibrinogen reached supranormal levels 2 days after the last sparsomycin dose. This, together with a tremendous increase in the number of young leukocytes, suggests an acute phase reaction, probably as a response to local damage to liver cells. The rapid recovery of plasma fibrinogen levels suggests a high priority for its synthesis, which may explain the suppression of albumin [19] and factor-VII synthesis.

On day 9, the mean plasma albumin levels had decreased by 8.5 g/l. The decrease was slower than that observed for factor VII and/or fibrinogen, and probably did not contribute to the toxic effect of sparsomycin. The disappearance $t_{½}$ was only a little lower than the supposed biological $t_{½}$ of albumin in humans [8].

The liver enzymes SGOT, SGPT, LDH, alkaline phosphatase, and γ-GTP exhibited moderately elevated levels during and after the treatment. However, it is difficult to differentiate the effect of sparsomycin from the effect of combined pentobarbital/sparsomycin toxicity. A spontaneous return to normal levels occurred within 10 days.

The decrease in creatinine clearance observed in all dogs might have been due primarily to prerenal factors. Dogs treated with sparsomycin vomited frequently and were unable to retain water after drinking. Additional subcutaneous hydration was insufficient to restore normal creatinine clearance levels.

During treatment with sparsomycin, no bone-marrow toxicity was observed. This is in accordance with the findings of earlier toxicological studies [14] and recent in vitro studies [5]. During the treatment course, we found decreased numbers of reticulocytes ($P = 0.01$).

In conclusion, our observations so far allow the following speculations about the pharmacokinetics and toxicity of sparsomycin. This drug is mainly eliminated by metabolic clearance (60%), and this may be inhibited or saturated after prolonged drug administration. This results in drug accumulation in plasma. Slow release of the drug from its binding sites and the enterohepatic circulation of sparsomycin in combination with renal-tubular resorption mechanisms may produce chronic, low plasma sparsomycin levels (below detection limits) and protracted toxicity. Acute 'auto-inhibition' of tubular excretion results in a decrease in renal drug clearance and, in the case of continuous infusion, increased drug plasma levels. The significance of this mechanism for drug toxicity is unknown. A decrease in the activity of liver microsomal enzymes (e.g., cytochrome P450) after prolonged sparsomycin administration might change the metabolism of other drugs ad-

ministered concomitantly with sparsomycin and thereaby potentiate their toxicity.

The dose-limiting toxicity for sparsomycin is liver toxicity and hemorrhage. The monitoring of plasma fibrinogen levels and thrombotest data should provide valuable indications of sparsomycin toxicity. Changes in factor-VII levels might be a useful dynamic parameter for showing the effect of sparsomycin on the host liver. The renal toxicity of sparsomycin should not be considered as dose limiting until prerenal factors have been definitively excluded.

In future studies a prolonged daily schedule of sparsomycin or its continuous infusion should be used with caution. To avoid drug accumulation without losing antitumor activity, the dose schedule should be spread out, and vigorous hydration should always be employed. Another possibility would be the introduction of more lipophilic sparsomycin analogs that are more easily eliminated by the liver, and not by the kidneys. Such analogs have recently been developed by our group [1, 10], and preliminary studies have indicated that they have favorable in vitro [1] and in vivo antitumor activity [26].

Acknowledgments. We wish to thank Dr. A. Pinckers for the expert fundoscopies. We would also like to thank the technicians of the Central Animal Laboratory of the University of Nijmegen (Head: Dr. W. van der Gulden) for their help during experiments. We thank Dr. I. Smale-Novakova, Mr. B. Verbruggen, and Mr. R. Polenwen from the Hematological Laboratory for factor-VII, fibrinogen, and thrombotest determinations, as well as their critical comments on this paper.

References

1. Broek vd LAGM, Liskamp RMJ, Colstee JH, Lelieveld P, Remacha M, Vazquez D, Ballesta JPG, Ottenheijm HCJ (1987) Structure-activity relationships of sparsomycin and its analogues. Inhibition of peptide bond formation in cell-free systems, and of L1210- and bacterial cell growth. J Med Chem 30: 325
2. Carter SK, Selawry O, Slavik M (1977) Phase I clinical trials. In: Saunders JF, Carter SK (eds) Methods of Development of new anticancer drugs. National Cancer Institute, Maryland, p 75
3. Close HP, McFarlane JR (1964) Ocular toxicity with sparsomycin (NSC 59729) in a phase I study: a preliminary report. Cancer Chemother Rep 43: 29
4. Cunha-Vaz J (1979) The blood-ocular barriers. Surv Ophtalmol 23: 279
5. Fiebig HH, Schmid JR, Köpping K, Ottenheijm HCJ, Wagener DJT, Zylicz Z, Löhr GW (1987) In vitro and in vivo anticancer activity of mitozolomide and sparsomycin in human tumor xenografts, murine tumors and human bone marrow. Cancer Res (in press)
6. Fodstad Ø, Pihl A (1982) Synergistic effect of ricin in combination with daunorubicin, cis-dichlorodiammineplatinum (II) and vincristine in systemic L1210 leukemia. Cancer Res 42: 2152
7. Hromas R, Barlogie B, Swartzendruber B, Drewinko B (1983) Potentiation of DNA -reactive antineoplastic agents and protection against S-phase-specific agents by anguidine in Chinese hamster ovary cells. Cancer Res 43: 3070
8. Jarnum S, Jensen KB (1978) Distribution and degradation of albumin. In: Yap S, Majoor CHL, van Tongeren JHM (eds) Clinical aspects of albumin. Martinus Nijhof, The Hague, p 81
9. Koj A (1974) Fibrinogen In: Allison AC (ed) Structure and function of plasma proteins. Plenum Press, New York, p 73
10. Liskamp RMJ, Colstee JH, Ottenheijm HCJ, Lelieveld P, Akkerman W (1984) Structure activity relationships of sparsomycin and its analoques. Octylsparsomycin: the first analogue more active than sparsomycin. J Med Chem 27: 301
11. Monro RE, Celma ML, Vazquez D (1969) Action of sparsomycin on ribosome-catalyzed peptidyl transfer. Nature 222: 356
12. National Cancer Institute, Bethesda, Maryland (1985) Screening data summary on sparsomycin (NSC-59729)
13. National Cancer Institute, Bethesda, Maryland (1963) Clinical brochure
14. National Cancer Institute, Bethesda, Maryland (1967) Toxicological report. Reported-dose intravenous toxicity of NSC 59729 (sparsomycin) in rats and monkeys including electroretinograph studies
15. Ottenheijm HCJ, Liskamp RMJ, van Nispen SPJM, Boots HA, Tyhuis MW (1981) Total synthesis of the antibiotic sparsomycin, a modified amino-acid monoxodithioacetal. J Org Chem 46: 3273
16. Owen SP, Dietz A, Camiener GW (1962) Sparsomycin, a new antitumor antibiotic. I. Discovery and biological properties. Antimicrob Agents Chemother 772
17. Owren PA (1964) Anticoagulants and their control in the surgical patient. Ann NY Acad Sci 115: 493
18. Poller L, Thomson JM, Sear CHJ, Thomas W (1971) Identification of a congenital defect of factor VII in a colony of beagle dogs: the clinical use of the plasma. J Clin Pathol 24: *626*
19. Princen JMG, Nieuwenhuizen W, Mol-Backx GPBM, Yap SH (1981) Direct evidence of transcirptional control of fibrinogen and albumin synthesis in rat liver during the acute phase response. Biochem Biophys Res Commun 102: 717
20. Schaefer FV, Theil EC (1981) The effect of iron on the synthesis and amount of ferritin in red blood cells during ontogeny. J Biol Chem 256: 1711
21. Sonenshein GE, Brawerman G (1976) Differential translation of mouse myeloma messenger RNAs in a wheat germ cell-free system. Biochemistry 15: 5501
22. Wagner JG (1979) Clinical pharmacokinetics. Drug Intelligence Publications, Hamilton Illinois, p 1
23. Winograd B, Oosterbaan MJM, vd Kleijn E, Liskamp RMJ, Ottenheijm HCJ, Wagener DJT (1983) Determination of sparsomycin in plasma and urine of the dog by means of reversed-phase high-performance liquid chromatography and first pharmacokinetic results. J Chromatogr 275: 145
24. Zähringer J, Baliga BS, Munro HN (1976) Novel mechanism for translational control in regulation of ferritin synthesis by iron. Proc Natl Acad Sci USA 73: 857
25. Zylicz Z, Wagener DJT, v Rennes H, Wessels JMC, vd Kleijn E, de Grip WJ, Ottenheijm HCJ, vd Broek LAGM (1986) In vivo potentiation of cis-diamminedichloroplatinum (II) antitumor activity by pretreatment with sparsomycin. Cancer Lett 32: 53
26. Zylicz Z, Wagener DJT, v Rennes H, vd Kleijn E, vd Broek LAGM, Ottenheijm HCJ. Antitumor activity of sparsomycin (NSC-059729) and its analogs. (Abstract). 5th EORTC and NCI Symposium on new drugs in cancer therapy, Amsterdam, 22–24 October, 1986, p 12
27. Zylicz Z, Wagener DJT, v Rennes H, Wessels JMC, vd Kleijn E, de Grip WJ, vd Broek LAGM, Ottenheijm HCJ (1987) In vitro modulation of cis-platin cytotoxicity by sparsomycin inhibition of protein synthesis. J Natl Cancer Inst 78: 701

Received December 1, 1986/Accepted April 15, 1987