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Preclinical toxicologic evaluation of DENSPM (N^1, N^{11}-diethylnorspermine) in rats and dogs

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A toxicology study of DENSPM was carried out in rats by multiple (once daily for 5 days) intravenous injection. Doses of 12.5, 25 and 50 mg DENSPM/kg were well tolerated. Infusion of 100 mg DENSPM resulted in distressing physical signs, including labored breathing, convulsive movements and acute death. There were no end-organ toxicities induced by this regimen as evaluated by serum chemistry and hematology examinations, and histopathologic exam of all major body organs. Transient hypotension was induced in rats and dogs by rapid infusion of DENSPM; the magnitude of this hypotension was decreased by slow infusion of DENSPM into dogs. Hypotension appears to be the dose-limiting toxicity of this agent when infused rapidly.

Key Words: Diethylnorspermine, dogs, hypotension, rats, toxicity.

Introduction

Polyamines are polycations that apparently play a role in cellular proliferation and differentiation.[1,2] Recently *N*-alkylated analogs of the naturally occurring polyamine, spermine, have been demonstrated to inhibit tumor growth in preclinical test systems.[3–5] The most effective of these analogs, N^1, N^{11}-diethylnorspermine (DENSPM),[4] has been shown to deplete intracellular polyamine pools by down-regulating the biosynthetic enzymes ornithine decarboxylase and S-adenosylmethionine decarboxylase, and by inducing the polyamine acetylating enzyme, spermidine/spermine N^1-acetyltransferase, which leads to increased polyamine catabolism and excretion.[4] Significant antitumor activity of DENSPM has been demonstrated against a number of human tumors grown in nude mice, including melanomas SH-1, MALME-3M and PANUT-3; A121 ovarian carcinoma; A549 lung adenocarcinoma; and HT29 colon carcinoma.[3–5]

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In light of the ease of synthesis of DENSPM, its excellent formulation and stability characteristics, and its strong activity in preclinical test systems, DENSPM is an excellent candidate for clinical trial. The purpose of the study reported here was to determine the organ specific toxicities of this promising agent prior to human phase I clinical trial.

Materials and methods

Materials

DENSPM was obtained from the Sunpharm Corporation (Gainesville, FL) as a lyophilized powder. The drug was dissolved in varying amounts of saline (0.9% NaCl) to achieve a final injection volume of 5 ml/kg body weight and used within 1 h of formulation. Rats (CD) were obtained from the Charles River Breeding Laboratories (Wilmington, MA), and maintained in wire mesh cages and fed water and rodent chow of 6% fat content (Teklad, Winfield, IA) *ad libitum*. Beagle dogs were obtained from Hazleton Research Products, (Cumberland, VA), maintained in individual runs and fed Iams (Dayton, OH) dog chow (300 g/day) and water *ad libitum*.

Toxicity studies in rats

Ten male and 10 female Charles River CD rats per experimental group (120 rats) were injected (tail vein) daily for five consecutive days with varying doses of DENSPM (12.5, 25, 50 and 100 mg DENSPM/kg; injection volume 5 ml/kg). Control groups included rats that received saline (5 ml/kg) and rats that were not injected. One half of the surviving animals were sacrificed the day after the final injection and the rest sacrificed 28 days later. Body weights were taken daily for the first 2 weeks and weekly thereafter until sacrifice. Clin-

ical symptoms were checked constantly during the 1 h period after injection and twice daily thereafter. At the time of sacrifice rats were deeply anesthetized with ether, the thoracic cavity opened and the rats exsanguinated by cardiac puncture. Individual hematology and serum chemistry exams were carried out on all animals. The hematology tests carried out consisted of erythrocyte, leukocyte, platelet and reticulocyte counts; white blood cell differential, and measurement of hemoglobin, hematocrit and erythrocyte indices (mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration). Serum chemistry parameters evaluated included glucose, BUN, creatinine, electrolytes (Na, K, Cl, P, Mg), cholesterol, bilirubin, serum enzymes (alkaline phosphatase, alanine and aspartate aminotransferase, lactate dehydrogenase, creatinine phosphokinase, amylase, gamma-glutamyl transferase), total protein and globulin. Analysis of variance (ANOVA) and Student's *t*-test were used for evaluation of statistical significance for body weight, hematology and clinical chemistry data.

At necropsy, organ weights (adrenals, kidneys, liver, spleen, testes and ovaries) were determined, and tissues removed, fixed in 10% formalin, embedded and sectioned, and stained with hematoxlin and eosin for histopathologic evaluation. The tissues examined included esophagus, stomach, duodenum, jejunum and ileum, colon, liver, pancreas, kidneys, urinary bladder, testes and epididymis or ovaries and uterus, diaphragm, femur, thyroid and parathyroid, salivary gland, axillary and mesenteric lymph nodes, trachea, brain, pituitary, mammary tissue, skin, thymus, heart, lung, adrenals, spleen, right eye, and the aorta.

Blood pressure studies in rats and beagle dogs

Male rats (440–560 g, 3–4 months of age) were anesthetized with sodium pentobarbital (50 mg/kg i.p.), the femoral vein or carotid artery isolated and cannulated with a 24 gauge pediatric outside the needle catheter (Exel, Culver City, CA). The catheter was connected to a physiological pressure transducer coupled to a Mennen Medical 741 cardiac monitor (Mennen Medical, Clarence, NY) set to measure mean blood pressure. Baseline blood pressures were established for a 5–10 min period followed by DENSPM infusion over a 15 s period via the cannulated lateral tail vein (one rat per dose level). Blood pressure recordings were taken every 30 s for the first 10 min after drug infusion and then every minute thereafter until 30 min after infusion. Mature male beagle dogs were anesthetized with sodium pentobarbital (to effect) and the femoral vein catheterized with an 18 gauge over the needle catheter (Deseret Pharmaceutical, Sandy, UT). DENSPM was infused at varying dosages (25, 50 or 100 mg/kg) over 15 s or 30 min time periods, and diastolic and systolic blood pressures measured as in rats.

Results

Toxicity studies in rats

A preclinical safety study of DENSPM was carried out in Charles River CD rats in comparison with a control group that received saline and a group that was not injected. All agents were given intravenously (the intended route in man) once daily for five consecutive days. Infusion time was approximately 30 s. The doses employed were 100, 50, 25 and 12.5 mg DENSPM/kg. The doses were based on a preliminary range finding study that revealed acute deaths (immediately post-injection in 6/6 animals) at 200 mg DENSPM/kg. The volume of saline in the control group was the same volume used for injection of DENSPM (5 ml/kg). A group of animals that received no injections was included to control for the stress of injection. Necropsy of the surviving animals was on day 5 (the day after the last injection) and day 32. Day 5 was chosen to allow evaluation of all potential histopathologic lesions that may have developed during treatment, and day 32 was chosen to permit potential for recovery of toxicity and possibly to detect lesions of delayed toxicity. The parameters used to evaluate the agent were clinical observations, body weights, hematology and serum chemistry analyses (obtained at necropsy), tissue weights, and gross and histopathologic exam of the major body organs.

Clinical observations. Rats that received 12.5, 25 or 50 mg DENSPM/kg tolerated treatment well and did not display any physical signs that were considered abnormal. Rats that received the highest dose studied, 100 mg/kg, were in severe distress immediately after injection. Abnormal physical signs included convulsive movements, sedation and labored breathing (Table 1). These signs appeared only during the 2 min period after injection and were short-lived with apparent full recovery within the 2 min post-infusion period. Five of the 10 male

Table 1. Number of rats with abnormal physical signs[a]

Treatment day	Convulsive movements	Sedation, labored breathing	Acute death[b]
Males			
0		6/10	1/10
1		7/9	2/9
2	1/7	6/7	1/7
3	2/6	6/6	
4	2/6	4/6	1/6
Females			
0		7/10	
1	2/10	6/10	
2	1/10	9/10	
3		9/10	
4		5/10	

[a]Male and female Charles River CD rats (10 of each sex) were given multiple (daily for five consecutive days) intravenous doses of 100 mg DENSPM/kg.
[b]Within 2 min of intravenous infucion of 100 mg DENSPM/kg.

rats died during this 2 min post-infusion period, while none of the female rats succumbed to treatment. Deaths occurred on days 0, 1, 2 and 4.

Body weights. Treatment with DENSPM did not result in weight loss in any animal either during the 5 day treatment period or during the 4 week recovery period. Of importance is that rats that survived the highest dose given, 100 mg DENSPM/kg, did not lose weight during the treatment or post-treatment periods. Figure 1 shows weight gains in male and female rats (recovery groups, i.e. not sacrificed immediately after treatment) during and after treatment.

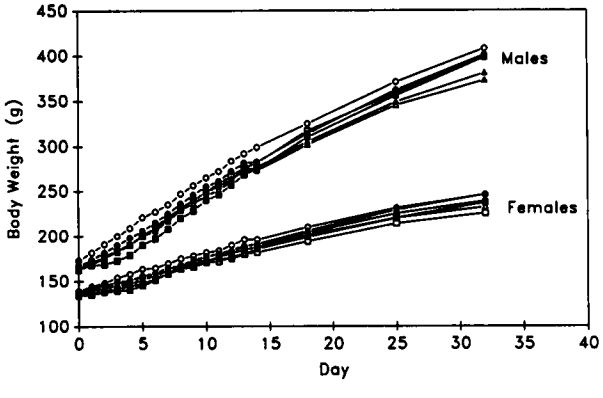

Figure 1. Effect of i.v. administration of DENSPM on rat body weights. Male and female CD rats (five rats/sex/group) were injected with 12.5 (△), 25 (▲), 50 (□) or 100 (■) mg DENSPM/kg, and body weights measured on days 0–14, 18, 25 and 32. Control groups were injected with saline (●) or received no injections (○).

Hematology. Blood taken at necropsy was evaluated for various erythrocyte parameters (i.e. hematocrit, hemoglobin, erythrocyte number, reticulocyte number, erythrocyte indices), myelocyte parameters (total WBC number and differential) and platelet number (Tables 2 and 3). A minor trend to decreased cell numbers was seen and included decreases in total WBC number (day 5, males and females), polymorph number (day 5, males and females) and lymphocyte number (day 5, females). However, analysis of variance revealed only one statistically significant difference ($p < 0.05$) between the treatment (female rats that received 50 mg DENSPM/kg, day 5 sacrifice, total WBC number) and control groups. There was a trend to increased hemotocrit/erythrocyte number in males and females receiving the highest dose, and sacrificed day 32. However, the findings are minor and of little toxicologic significance.

Serum chemistry. Serum chemistry evaluations were carried out on blood samples taken at necropsy with analysis of variance performed on all treatment groups for each parameter, in comparison with the saline injected rats and control rats that were not injected. There were no findings of toxicologic significance.

Gross necropsy. The only gross lesions seen were bladder concretions (probably proteinaceous in nature). These concretions were seen in the saline treated groups, and in the rats that received 12.5, 25 and 50 mg DENSPM/kg. These findings are of no apparent toxicologic significance.

Table 2. Erythrocyte and myelocyte parameters[a] of male CD rats determined at necropsy[b]

	RBC	HGB	WBC	Poly	Lymph	Platelet
Day 5 sacrifice						
no treatment	5.96	13.7	5.6	1.28	4.13	1178
±SD	0.23	0.7	1.5	0.58	0.95	136
saline	5.60	13.2	7.0	1.24	5.59	1205
±SD	0.19	0.4	2.2	0.53	2.39	57
12.5 mg DENSPM/kg	5.91	13.7	7.8	1.72	5.94	1322
±SD	0.28	0.5	2.2	0.97	1.40	198
25 mg DENSPM/kg	6.14	13.8	8.5	1.94	6.92	1256
±SD	0.26	0.4	1.8	0.81	2.08	129
50 mg DENSPM/kg	6.04	13.6	5.8	0.81	4.96	1284
±SD	0.45	0.2	2.3	0.26	2.18	100
100 mg/DENSPM/kg	6.14	14.4	5.6	0.90	4.57	1253
±SD	0.12	0.1	1.5	0.08	1.38	42
Day 32 Sacrifice						
no treatment	7.12	15.2	7.6	1.09	6.47	932
±SD	0.39	0.7	3.0	0.57	2.85	115
saline	7.14	15.0	9.7	1.33	8.15	1024
±SD	0.08	0.3	2.7	0.75	1.92	74
12.5 mg DENSPM/kg	7.03	15.3	10.7	1.19	9.39	1004
±SD	0.28	0.4	2.4	0.52	2.12	95
25 mg DENSPM/kg	7.39	15.6	10.1	1.56	8.38	990
±SD	0.17	0.3	2.6	0.64	2.27	78
50 mg DENSPM/kg	7.44	15.4	10.3	1.39	8.82	1117
±SD	0.14	0.2	2.3	0.88	1.81	76
100 mg/DENSPM/kg	7.97*	16.2	9.1	1.55	7.5	1042
±SD	0.26	0.5	0.4	0.42	0.61	64

[a] Units are: RBC (erythrocyte number) (millions/cubic millimeter); HGB (hemoglobin) (g/dl); WBC (total myelocyte number) (thousands/cubic millimeter); Poly (neutrophil number) (thousands/cubic millimeter); Lymph (lymphocyte number) (thousands/cubic millimeter); Platelet (platelet number) (thousands/cubic millimeter).

[b] Male Charles River CD rats (five rats/ sacrifice group) were injected with 12.5, 25, 50 or 100 mg DENSPM/kg by a multiple dose schedule (daily for 5 consecutive days). Saline injected rats received 5 ml saline/kg/day. Control rats were not injected.

* $p < 0.05$.

Tissue weights. At necropsy, weights of various tissues were examined (adrenals, kidneys, liver, spleen, testes, ovaries) and an analysis of variance carried out on all four groups of treated animals. Only the right adrenal mean weights in male rats (day 32 sacrifice) were significantly different from controls. Because there were no apparent adrenal weight differences in other animal groups, lack of histopathologic lesions of the adrenals and no differences in the left adrenal weights of the same animals, it is unlikely that this finding is of any toxicologic significance.

Histopathology. All rats were necropsied and all tissues from all rats were examined for histopathologic lesions. A wide variety of lesions were seen in the necropsied rats. The type of lesion seen, the time seen (day 5 or 32) and in which groups are shown in Table 4. The lesions were considered to be background in nature and not drug related.

Blood pressure studies in rats

A pilot study was carried out to determine if severe hypotension may have been the cause of the clinical signs shown by the animals that received 100 mg DENSPM/kg (i.e. sedation, labored breathing). Four male rats were anesthetized with sodium pentobarbital and an artery catheterized to permit blood pressure measurements with a cardiac monitor. The pretreatment blood pressure readings were 103, 105, 112 and 104 Torr for the animals that received 12.5, 25, 50 and 100 mg DENSPM/kg, respectively. One animal per dose was treated. Results of the individual experiments are shown in Figure 2. Infusion over 15 s of DENSPM resulted in an immediate fall in median blood pressure, with nadir values reached by 30 s. A dose–response relationship is apparent, i.e. greater falls in blood pressure occurred with increasing doses of DENSPM. The lowest mean blood pressure re-

Table 3. Erythrocyte and myelocyte parameters[a] of female CD rats determined at necropsy[b]

	RBC	HGB	WBC	Poly	Lymph	Platelet
Day 5 sacrifice						
no treatment	6.17	14.2	6.6	0.85	5.67	1042
±SD	0.41	0.5	2.3	0.47	1.85	54
saline	5.69	13.3	6.6	0.88	5.48	1249
±SD	0.65	0.9	4.7	0.85	3.67	226
12.5 mg DENSPM/kg	5.89	13.8	5.7	1.41	4.24	1291
±SD	0.26	0.3	1.8	0.76	1.15	145
25 mg DENSPM/kg	6.05	14.2	5.0	0.78	4.06	1088
±SD	0.13	0.3	1.3	0.41	1.01	226
50 mg DENSPM/kg	6.12	14.1	4.0*	0.49	3.37	1078
±SD	0.31	0.1	1.8	0.26	1.54	273
100 mg/DENSPM/kg	6.37	14.0	4.3	0.63	3.58	1238
±SD	0.19	0.4	1.1	0.25	1.00	58
Day 32 sacrifice						
no treatment	7.02	15.5	6.1	0.80	5.32	890
±SD	0.08	0.4	1.7	0.56	1.26	90
saline	7.12	15.1	5.4	0.53	4.78	925
±SD	0.24	0.4	1.1	0.33	0.75	103
12.5 mg DENSPM/kg	7.00	15.0	6.0	0.86	5.05	907
±SD	0.38	0.7	2.0	0.50	1.64	147
25 mg DENSPM/kg	7.27	15.4	6.1	0.58	5.47	1051
±SD	0.19	0.3	1.3	0.24	1.04	71
50 mg DENSPM/kg	7.75*	15.8	6.7	0.91	5.73	786
±SD	0.27	0.6	1.2	0.20	1.22	230
100 mg/DENSPM/kg	7.46*	15.4	6.8	0.68	6.06	857
±SD	0.11	0.3	1.1	0.18	0.98	146

[a] Units are: RBC (erythrocyte number) (millions/cubic millimeter); HGB (hemoglobin) (g/dl); WBC (total myelocyte number) (thousands/cubic millimeter); Poly (neutrophil number) (thousands/cubic millimeter); Lymph (lymphocyte number) (thousands/cubic millimeter); Platelet (platelet number) (thousands/cubic millimeter).

[b] Female Charles River CD rats (5 rats/ sacrifice group) were injected with 12.5, 25, 50 or 100 mg DENSPM/kg by a multiple dose schedule (daily for 5 consecutive days). Saline injected rats received 5 ml saline/kg/day. Control rats were not injected.

* $p < 0.05$.

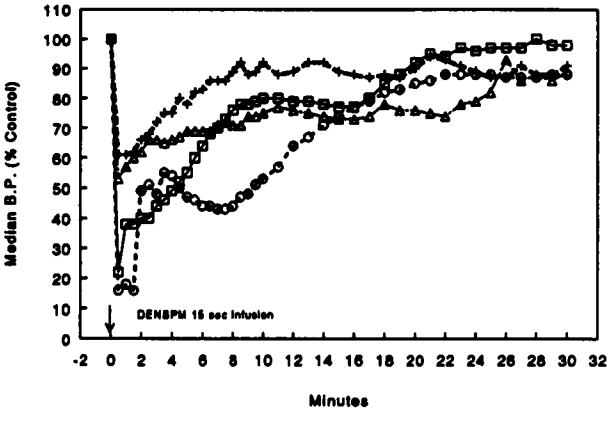

Figure 2. Effect of i.v. administration of DENSPM on mean arterial blood pressure in anesthetized rats. DENSPM at doses of 12.5 (+), 25 (△), 50 (□) or 100 (○) mg/kg was infused over a 15 s time period, and median arterial pressure recorded with a cardiac monitor as detailed in text.

corded was 12 Torr in the rat that received 100 mg DENSPM/kg. Recovery from hypotension was rapid and nearly complete by 30 min after infusion. It is not clear if failure to completely return to pretreatment values is a function of the length of anesthesia or possibly lingering drug effects.

Blood pressure studies in beagle dogs

Studies were conducted in another species (beagle dogs) to confirm the hypotensive properties of DENSPM. Dogs were anesthetized, the femoral artery cannulated, and baseline values for diastolic and systolic blood pressure established. One dog was infused with 25 mg DENSPM/kg over a 30 s time period (Figure 3). The diastolic and systolic blood pressures fell by over 50%, with recovery

Table 4. Summary of histopathologic lesions[a,b]

	Control	Saline	mg DENSPM/kg			
			12.5	25	50	100
Lung						
round cell foci			+			
granulomas		+				
focal hemorrhage						+
vascular hypertophy					+	
Kidney						
tubular regeneration	*		**	+		
round cell foci		+*	*	*		++*
inflammatory cell focus	*					
hydronephrosis	+*	*	+**			
muscularis proliferation			+			
fibrosis				+		
chronic pyelitis						+
Liver						
round cell foci	+*	++**	++	+++	+++++*	++++
bile duct proliferation	*			++	+	+++
Heart						
round /inflammatory cell	*					
Bladder						
concretion		**	++	+*	+++**	
chronic cystitis						+
epithelial hyperplasia						+
Ileum						
lymphoid hyperplasia		*				
Colon						
crypt loss				*		
focal colitis				*		
Lymph node						
hyperplasia	+		*			
sinus histiocytosis		*				
Cerebellum						
hemorrhage				+		
Thymus						
focal hemorrhage					+	
congestion						+
Testes						
atrophy				*		
spermatic granulation tissue				*		

[a] Male and female (five/group/sex) Charles River CD rats were injected with 12.5, 25, 50 or 100 mg DENSPM/kg by a multiple dose schedule (daily for five consecutive days). Saline injected rats received 5 ml saline/kg/day. Control rats were not injected.
[b] Each '+' represents one rat with the indicated lesion (day 5 autopsy); a '*' represents a rat with

to normal within 30 min. The dog received a supplementary dose of anesthesia (at 45 min) and reinfused 15 min later. The magnitude of the fall in blood pressure was similar to that after the first infusion. Recovery was nearly complete by 9 min after the infusion, but never fully recovered to pretreatment levels. The same dog was infused with the same amount of DENSPM 72 h later (Figure 4); however, the infusion period was extended to 30 min. The magnitude of fall in diastolic/systolic pressures was much smaller (5–20%) than after rapid (30 s) infusion. Anesthesia was supplemented 40 min after the start of the infusion and the dog was reinfused with a 25 mg DENSPM/kg dose over 30 s. The magnitude of the falls in diastolic and systolic pressure were similar to those seen 3 days previously (approximately 60%) and again recovery was rapid, but incomplete. Another dog (Figure 5) was infused over a 30 min time period with 50 mg DENSPM/kg. The magnitude of fall in blood pressures was greater than after 25 mg DENSPM/kg (approximately 35%). The dog was reinfused

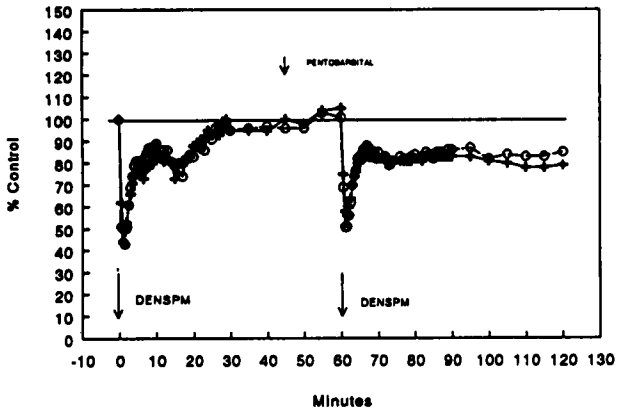

Figure 3. Effect of i.v. administration of DENSPM on diastolic (+) and systolic (O) blood pressure in an anesthetized beagle dog. DENSPM (25 mg/kg) was infused over a 30 s time period and reinfused (over 30 s) 60 min later. Pentobarbital anesthesia was supplemented 45 min after the first injection (arrow).

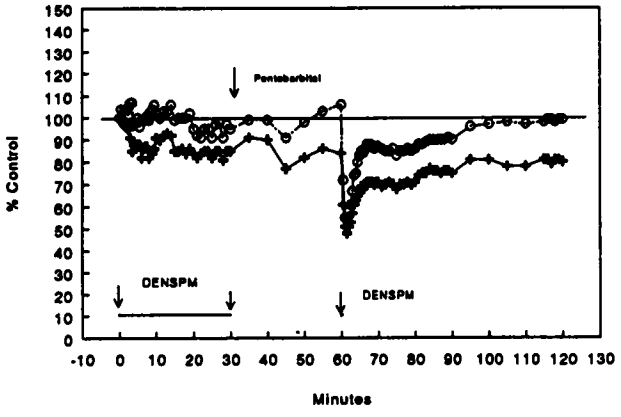

Figure 4. Effect of i.v. administration of DENSPM on diastolic (+) and systolic (O) blood pressure in an anesthetized beagle dog. DENSPM (25 mg/kg) was infused over a 30 min time period and reinfused (over 30 s) 30 min later. Pentobarbital anesthesia was supplemented after the first injection (arrow).

(50 mg DENSPM/kg, 30 s infusion) 1 h after the beginning of the first infusion. Again a rapid but incomplete recovery from hypotension was documented. The dose of DENSPM was doubled to 100 mg/kg and given by rapid (30 s) intravenous infusion. Diastolic and systolic blood pressures fell rapidly, stabilized, but started to fall again 4–5 min after infusion (Figure 6). Within 8–9 min after infusion the dog died. It should be noted that this dose (100 mg DENSPM/kg) of drug was the same amount of drug given as a cumulative dose over 1 h to the previous dog (two infusions of 50 mg DENSPM/kg).

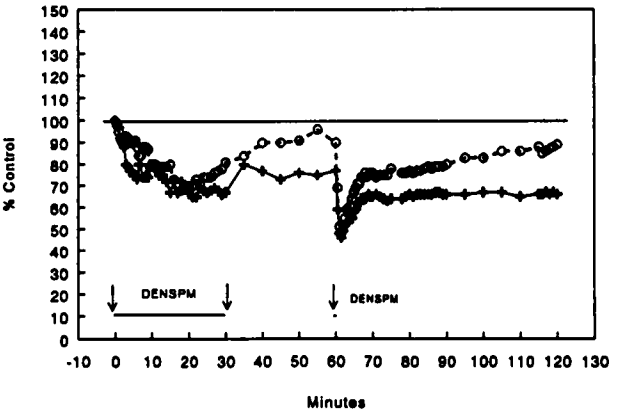

Figure 5. Effect of i.v. administration of DENSPM on diastolic (+) and systolic (O) blood pressure in an anesthetized beagle dog. DENSPM (50 mg/kg) was infused over a 30 s time period and reinfused (over 30 s) 30 min later.

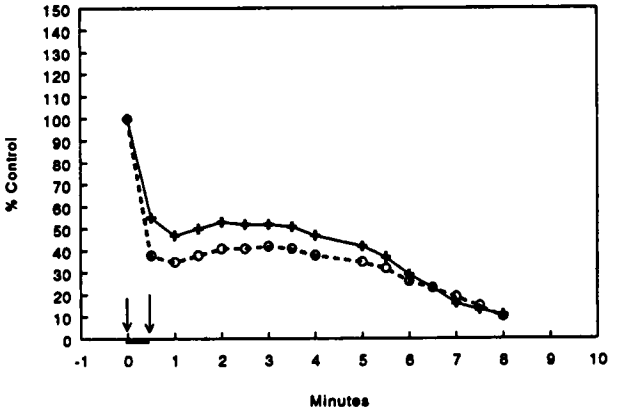

Figure 6. Effect of i.v. administration of 100 mg DENSPM/kg on diastolic (+) and systolic (O) blood pressure in an anesthetized beagle dog.

Discussion

The present study was carried out to determine the toxicities induced by DENSPM when given in large, single daily doses to rats for five consecutive days. The primary toxicities noted were distressing physical signs and lethality in rats given the highest dose possible, 100 mg/kg. Previous range-finding studies not reported here established that 200 mg DENSPM/kg, given intravenously by rapid infusion, was always lethal (6/6 animals treated with 200 mg DENSPM/kg died within minutes of injection). We could detect no significant drug-induced end-organ toxicities in rats as evaluated by serum chemistry and hematology tests, body weight measurements, and histopathologic exam. Importantly, there was little evidence for significant dose-related myelosuppression, the most common toxicity of the currently available antineoplastic agents. It is possible, how-

ever, that myelosupression may be detected after different dose regimens (e.g. constant infusion or multiple daily doses), with evaluations at different time periods after dosage than were employed in this study. The lack of myelosupressive activity reported here is consistent with the findings in dogs treated three times daily for 6 days with the maximally tolerated dose of DENSPM (personal communication, Sunpharm). In the toxicity study conducted in dogs gastrointestinal toxicity was dose-limiting when DENSPM was given three times daily by slow (15 min) infusion. Gastrointestinal toxicity, as manifested by clinical signs (e.g. diarrhea, etc.), serum chemistry alterations or histopathologic lesions, was not evident in the rat study reported here.

The physical signs and acute deaths suffered by rats receiving the highest doses of DENSPM were suggestive of cardiovascular toxicity. To evaluate this possibility, rats were placed under general anesthesia, injected with DENSPM and mean arterial pressure monitored. A transient, dose-related decrease in mean arterial pressure was measured in the rats. Nadir values were reached within 30 s of DENSPM infusion, with subsequent rapid recovery. In the rat treated with a potentially lethal dose of DENSPM (100 mg/kg), median blood pressure fell from 104 to 12 Torr. This profound drop could explain the lethal events in male rats that received this dose. Studies are currently underway in rats to determine if there is a sex difference in DENSPM-induced hypotension.

DENSPM-induced hypotension was confirmed in dogs. Injection of 25 mg DENSPM/kg resulted in a transient but significant decrease in arterial blood pressure. Reinfusion shortly thereafter in the same dog elicited a hypotensive response of the same magnitude. Recovery to normal blood pressure levels did not occur after the second dose, but it is not clear if this finding was secondary to anesthesia, rather than drug accumulation. Slow (30 min) infusion of the same dose resulted in little hypotension. However, slow infusion (30 min) of a higher (50 mg/kg) dose of DENSPM resulted in significant hypotension. Rapid infusion of 100 mg/DENSPM resulted in the death of the one dog that received this dose, apparently from profound hypotension. It is probably significant that the dog that received the same dose over a 1 h time period (50 mg by two infusions, one slow and one rapid) did not die.

Based on these findings, it would appear prudent and reasonable that careful blood pressure monitoring be carried out in humans receiving this agent and that the agent be given by slow infusion, rather than by bolus intravenous injection.

The hypotensive effects demonstrated here are not unexpected in light of a recent literature report[6] which showed that infusion of positively charged polyamines into rats resulted in a transient decrease in mean arterial pressure, with recovery within minutes of infusion. The basis for this hypotension was an apparent polyamine-induced decrease in intracellular calcium concentration of vascular smooth muscle, resulting in relaxation of the vasculature. The effect was reversed both *in vitro* and *in vivo* by administration of calcium salts.

In recent years other inhibitors of polyamine synthesis have been developed to clinical trial. Eflornithine–HCl (α-difluoromethylornithine or DFMO) is an irreversible inhibitor of ornithine decarboxylase, with demonstrated activity in preclinical tests systems such as mouse mammary EMT 6 sarcoma, murine leukemia L1210 and Lewis lung carcinoma,[7–9] and human small cell carcinoma grown in nude mice.[10] In clinical trials the major toxicities have been shown to be thrombocytopenia (the dose-limiting toxicity), anorexia, gastrointestinal toxicity and hearing loss.[11,12] Clinical utility has been disappointing due probably to the limited inhibitory potency of DFMO[3] and the need for prolonged (many weeks) treatment as demonstrated in preclinical test systems.[10]

Methylglyoxal-bis(guanylhdrazone) (methyl-GAG) shares some structural activity with the naturally occurring polyamines and has been extensively tested in clinical trial.[14] While the exact cytotoxic mechanism of action is unclear, numerous studies suggest that the mechanism of action is inhibition of polyamine biosynthesis.[15] In early clinical trials employing repetitive daily administration, the agent was shown to produce severe toxicities.[15] Pharmacologic studies showed that frequent dosing resulted in drug accumulation and therefore the potential for severe toxicity.[17] Increased tolerance was noted with less frequent dosage schedules[18] and the agent has been shown to have substantial clinical activity against malignant lymphoma.[19]

Given the favorable toxicity profile of DENSPM, its established preclinical efficacy in murine tumor systems and clinical success with other polyamine inhibitors (e.g. methyl-GAG), DENSPM appears to be an excellent candidate for human clinical (phase I) trial. Phase I clinical trial of DENSPM is currently underway at Roswell Park Cancer Institute.

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