

Transgenic Mice with Activated Polyamine Catabolism due to Overexpression of Spermidine/Spermine N^1 -Acetyltransferase Show Enhanced Sensitivity to the Polyamine Analog, N^1,N^{11} -Diethylnorspermine

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Received September 30, 1998; accepted December 18, 1998

This paper is available online at <http://www.molpharm.org>

ABSTRACT

We have recently generated transgenic mice in which polyamine catabolism has been activated by overexpressing the rate-limiting enzyme of polyamine catabolism, spermidine/spermine N^1 -acetyltransferase (SSAT). These animals have now been tested for their sensitivity to the polyamine analog N^1,N^{11} -diethylnorspermine (DENSPM), which is currently undergoing Phase I clinical trial. The analog is known for its ability to potentially induce SSAT. Treatment for 4 days with a daily dose (125 mg/kg) of analog caused profound changes in polyamine metabolism in the transgenic animals. Liver SSAT activity was increased by approximately 800-fold while hepatic mRNA increased only 4-fold. Putrescine pools increased while spermidine and spermine pools nearly disappeared, resulting in a compensatory increase in ornithine decarboxylase activity. Similar but less profound changes were also seen in other

tissues (spleen, intestine, and skin). This treatment also resulted in a 50% mortality in the transgenic animals, with no apparent histopathological changes in major organs. Nontransgenic animals exhibited no toxicity, and tissue SSAT activity was unchanged or only moderately increased. Polyamine pools were only slightly altered. Greater analog toxicity in transgenic animals may be attributable to higher tissue levels of DENSPM facilitated by SSAT-mediated decreases in spermidine and spermine. To further confirm the enhanced sensitivity of the transgenic animals to the analog, groups of nontransgenic and transgenic animals were subjected to daily injections with DENSPM. On average, transgenic mice died ~3 days earlier than their nontransgenic litter-mates. The findings indicate a contributing role for SSAT in whole animal toxicity by SSAT-inducing polyamine analogs.

The polyamine biosynthetic pathway has long been considered a worthwhile target for cancer chemotherapy. However, despite the impressive preclinical activity of inhibitors of ornithine decarboxylase (ODC), the rate-controlling enzyme of the biosynthesis, the results of clinical trials have so far not met expectations (Jänne et al. 1991; Porter et al. 1992; Kramer, 1996). Certain of these inhibitors are currently being reevaluated as chemopreventive agents (Creaven et al. 1993; Love et al. 1993), and promising results are emerging. On the basis of impressive preclinical findings (Regenass et al. 1992, 1994), a newly identified inhibitor of another biosynthetic enzyme, *S*-adenosylmethionine decarboxylase

(AdoMetDC), is now being evaluated in patients with solid tumors.

Another approach to cancer chemotherapy involves the use of *N*-alkylated polyamine analogs to deregulate polyamine biosynthesis and metabolism. Although initially developed to suppress the biosynthetic enzymes (Porter and Bergeron, 1988), subsequent studies revealed that the antiproliferative action of the analogs may be more closely linked with a striking activation of polyamine catabolism and/or excretion (Porter et al. 1992; Kramer, 1996). Polyamine analogs such as N^1,N^{11} -diethylnorspermine (DENSPM) have been shown to potentially induce spermidine/spermine N^1 -acetyltransferase (SSAT) (Casero et al. 1989; Pegg et al. 1989; Porter et al. 1991; Shappell et al., 1992), the enzyme that facilitates polyamine export and back-conversion via oxidative catabolism (Seiler, 1987). Studies with different human tumor cell lines (Casero et al. 1989; Porter et al. 1991; Casero et al. 1992;

This work was supported in part by the CA-65942 and CA-22153 US National Cancer Institute, National Institutes of Health, by the Academy of Finland and by Human Frontier Science Program.

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ABBREVIATIONS: DENSPM, N^1,N^{11} -diethylnorspermine; SSAT, spermidine/spermine N^1 -acetyltransferase; ODC, ornithine decarboxylase; AdoMetDC, *S*-adenosylmethionine decarboxylase.

Shappell et al. 1992) have revealed a close relationship between the antiproliferative action and the induction of SSAT. This linkage seems to be mediated by the depletion of spermidine and spermine, as facilitated by SSAT, together with the inability of the analog to substitute for the missing polyamines in functions required for cell proliferation. Indeed, substantial induction of SSAT without apparent cytotoxicity can be achieved with various methyl derivatives of spermine, which seem to be at least partially able to support cell growth (Yang et al. 1995; Kramer et al. 1997).

Very little is known about the consequences of constitutive overexpression of SSAT. Inducible expression of human SSAT in *Escherichia coli* apparently results in a reduced growth rate due to the conversion of all spermidine to N^1 -acetylspermidine (Parry et al. 1995b). The gene has also been transiently expressed in COS cells, resulting in an enhanced accumulation of putrescine, the appearance of N^1 -acetylspermidine, a decrease in spermidine and spermine pools, and a compensatory increase in biosynthetic enzyme activities (Vargiu and Persson, 1994). The fact that no effects on cell growth were reported is apparently attributable to the short-term nature of the experiment (Vargiu and Persson, 1994).

We recently generated a transgenic mouse line that constitutively overexpressed the murine SSAT gene in all tissues (Pietilä et al. 1997). Members of the transgenic line showed profound changes in tissue polyamine pools, including a massive accumulation of putrescine, the appearance of N^1 -acetylspermidine, and a decrease in spermidine and/or spermine pools (Pietilä et al. 1997). The phenotypic features of the transgenic animals included permanent hair loss at early age, skin wrinkling, and female infertility (Pietilä et al. 1997). In the present study, we have used these mice to examine the biological and metabolic consequences resulting from DENSPM induction of SSAT. Subchronic exposure of the transgenic animals to the drug led to a dramatic increase in SSAT activity and a near-total depletion of tissue spermidine and spermine pools, which were clearly related to activated polyamine catabolism. These changes were associated with greater analog toxicity in the transgenic animals.

Experimental Procedures

Materials. The spermine analog, DENSPM, was synthesized as described earlier (Bergeron et al. 1988, 1989) and was kindly provided by Warner Lambert Parke-Davis (Ann Arbor, MI). The drug was dissolved in saline and administered i.p. to mice.

Generation of Transgenic Mice. The transgenic mice were generated using the standard pronuclear microinjection technique (Hogan et al. 1986). Fertilized oocytes were obtained from BALBc × DBA/2 (CD2F1) females mated with CD2F1 males. The SSAT gene construct used was an 18-kbp genomic sequence isolated from 129 SVJ mouse genomic library (Stratagene, La Jolla, CA). The transgene construct contained all the exons and introns of the murine SSAT gene together with 3 kbp of the 5'-flanking region (endogenous promoter) and 11.5 kbp of the 3' flanking region. (Pietilä et al. 1997). Because the transgene was an endogenous mouse gene, the initial detection of transgenic animals with polymerase chain reaction was based on simultaneous integration of multiple transgene copies in the form of concatamers, so that a minimum of two integrated transgene copies were required for the detection (Pietilä et al. 1997). The detection of transgenic pups was later based on the permanent loss of hair at the age of 3 to 4 weeks. Transgenic status was also occasionally checked from DNA samples. The transgenic animals

used in this study were members of the line UKU165b, harboring more than 20 SSAT copies in their genome (Pietilä et al. 1997).

Analytical Methods. Total RNA was extracted with guanidine isothiocyanate (Chomczynski and Sacchi, 1987) and purified by CsCl gradient centrifugation (Ross, 1976). Human SSAT cDNA probe (Xiao et al. 1991) was used in Northern blot analyses. The activities of SSAT, ODC, and AdoMetDC were assayed as described earlier (Bernacki et al. 1995). Polyamines, their acetylated derivatives, and DENSPM were measured by HPLC as described by Kramer et al. (1995). The tissue specimens for histology were fixed in 10% formalin in phosphate buffer, embedded in paraffin, cut into 5- μ m sections, stained with hematoxylin/eosin, and evaluated microscopically. For statistical analyses, two-tailed *t* test was used.

Results

Effect of a Single Dose of DENSPM on Nontransgenic and SSAT Transgenic Mice. We have previously published a detailed analysis of baseline SSAT mRNA and activities in the various tissues of SSAT transgenic and nontransgenic animals (Pietilä et al., 1997). In an analysis of six tissues, we found that SSAT mRNA levels were elevated to a greater extent than SSAT activity, suggesting the existence of feedback regulation by polyamine pools via post-transcriptional mechanisms. In the present study, we initially examined the effects of the polyamine analog DENSPM on SSAT mRNA and activity in transgenic and nontransgenic animals. In a pilot experiment, mice (two mice in each group) received a single i.p. dose (100 mg/kg) of DENSPM 24 h before sacrifice. The analog was well-tolerated by transgenic and nontransgenic animals alike. DENSPM concentration was approximately 14-fold higher in transgenic liver (999–1032 pmol/mg tissue) in comparison with nontransgenic. Figure 1 depicts SSAT mRNA and activity from selected tissues (liver, kidney, brain, and intestine) of nontransgenic and transgenic animals, untreated or treated with DENSPM. In either transgenic or nontransgenic animals, the single injection

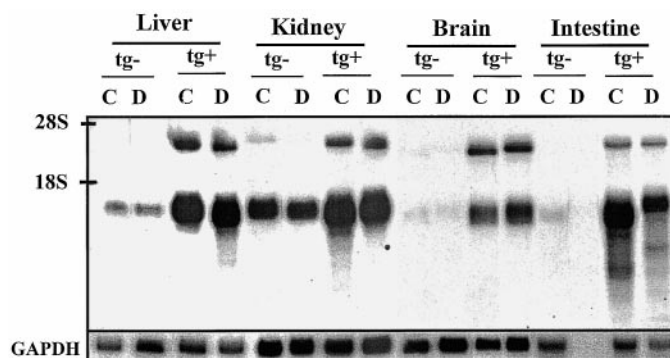


Fig. 1. Northern blot analysis of total RNA (20 μ g) isolated from different tissues of nontransgenic and transgenic animals untreated or treated with DENSPM. The animals received a single injection of DENSPM (100 mg/kg) 24 h before being euthanized (abbreviations: tg-, nontransgenic; tg+, transgenic; C, nontreated; D, DENSPM). 28S and 18S indicate the migration of 28S rRNA and 18S rRNA). The larger band (between 28S and 18S rRNA) is derived from the putative heteronuclear SSAT RNA and the smaller band from the mature SSAT mRNA. Signal intensities of SSAT mRNA bands were quantitated by densitometric scanning of the original films and standardized to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) signal as an internal control for lane-loading. Note tg- intestine samples have undergone partial mRNA degradation as indicated by ethidium bromide staining of the membrane (not shown) and the reduced GAPDH signal. SSAT activity (expressed as pmol/mg protein/min) is provided at the bottom of the Northern blot. The latter data represents mean values based on two animals per group.

TABLE 1

Effect of DENSPM on polyamine enzyme activities and pools in nontransgenic and transgenic animals^a

Tissue/treatment	Enzyme activity				Polyamine pools			
	ODC (<i>pmol/mg protein/h</i>)	AdoMetDC (<i>pmol/mg protein/h</i>)	SSAT (<i>pmol/mg protein/min</i>)	Put	Ac-Spd	Spd (<i>pmol/mg tissue wet weight</i>)	Spm	DENSPM
Liver (tg ⁻)	9 ± 5	692 ± 288	8 ± 0.4	16 ± 6	<5	942 ± 79	930 ± 58	
Liver (tg ⁻) + DENSPM	88 ± 73	875 ± 292	12 ± 3	167 ± 94	6 ± 1	592 ± 22	671 ± 87	467 ± 90
Liver (tg ⁺)	180 ± 20*	813 ± 142	20 ± 7*	543 ± 190*	62 ± 12***	870 ± 105	420 ± 38***	
Liver (tg ⁺) + DENSPM	439 (503,374)	558 (546,569)	16670 (21368,11978)	859 (632,1086)	37 (27,48)	12 (12,12)	10 (15,4)	2225 (2108,2341)
Spleen (tg ⁻)	0.5 ± 0.2	19 ± 5	43 ± 4	87 ± 14	<5	1226 ± 88	921 ± 95	
Spleen (tg ⁻) + DENSPM	6 ± 4	23 ± 3	282 ± 44	282 ± 111	9 ± 3	759 ± 164	623 ± 129	295 ± 138
Spleen (tg ⁺)	23 ± 7	57 ± 15*	84 ± 21*	2760 ± 517*	221 ± 87*	811 ± 126**	669 ± 64*	
Spleen (tg ⁺) + DENSPM	123 (17,228)	51 (38,63)	3240 (3808,2673)	4085 (3720,4451)	328 (367,288)	182 (135,228)	144 (138,150)	795 (921,669)

^a The animals received 125 mg/kg of diethylnorspermine on four consecutive days and were euthanized on the fifth day. There were initially three animals in control groups and four animals in the treatment groups, but only two survived of the transgenic group receiving the drug. Means ± standard deviations. * $P < .05$; ** $P < .01$; *** $P < .001$ (refers to the statistical differences between untreated nontransgenic and transgenic groups). Put, putrescine; Ac-Spd, N1-acetylspermidine; Spd, spermidine; Spm, spermine; tg⁻, nontransgenic; tg⁺, transgenic.

tion of the analog did not appear to increase SSAT mRNA levels by more than 2-fold and actually decreased them slightly (i.e., <30%) in the intestine. Analog treatment had a similar effect on SSAT activity in the tissues of nontransgenic animals, while increasing that of transgenic animals by up to 9.5-fold in the case of the liver, suggesting post-transcriptional stimulation. Consistent with the latter finding, the livers of the treated transgenic animals showed distinct signs of enhanced polyamine catabolism as indicated by a more than 20% decrease in spermidine and spermine pools and a 2.3-fold increase in putrescine. A more detailed analysis of analog effects was conducted in mice receiving multiple doses of analog as follows.

Effect of Multiple Doses of DENSPM on Nontransgenic and SSAT Transgenic Mice. Mice were subsequently subjected to daily treatment with higher multiple daily doses (200 mg/kg) of DENSPM. While the nontransgenic animals tolerated DENSPM without signs of toxicity, one of three transgenic animals in the treatment group died on day 3 and the remaining two animals appeared unwell and/or moribund. Thus, although the experiment was designed to involve 5 injections, treatment was terminated 24 h after the third injection. A histological examination of liver, spleen, kidney, heart, brain, skin, and small intestine of all drug-exposed animals failed to reveal signs of overt pathological changes, with the possible exception of an increase in the number of binuclear cells in the livers of DENSPM-treated transgenic animals. This occurred, however, in the absence of apparent necrosis or inflammation.

In an attempt to reduce mortality, animals were administered daily injections of DENSPM at a reduced dose level (125 mg/kg). Although this treatment was designed to last 5 days, the SSAT transgenic animals were again found to be more sensitive to the toxic effects of DENSPM. After the fourth injection, two of four animals died, and the experiment was terminated 24 h later. The causes of death were not obvious, and macroscopic examination revealed no signs of major organ involvement. A careful histological examination

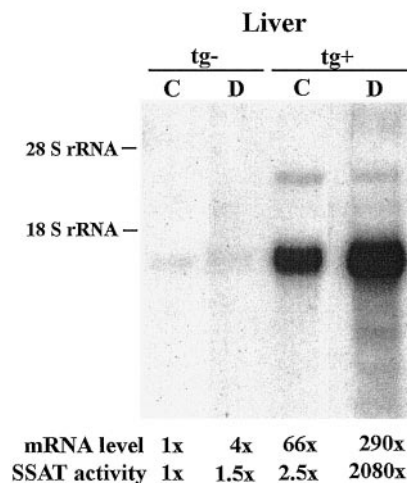


Fig. 2. Northern blot analysis of total RNA (20 µg) isolated from liver of nontransgenic and transgenic animals untreated or treated with DENSPM. The animals received daily injections (125 mg/kg) of DENSPM for 4 consecutive days and were euthanized 24 h after the last injection (abbreviations: tg⁻, nontransgenic; tg⁺, transgenic; C, nontreated; D, DENSPM). The relative amounts of mRNA and relative SSAT activities are given at the bottom of the figure. Signal quantitation was determined as in Fig. 1.

of liver, the tissue with the highest levels of SSAT activity, failed to reveal any overt pathological changes. There were, however, signs of gastrointestinal bleeding in the analog-treated transgenic animals but no obvious changes in mucosal histology.

This 4-day treatment schedule gave rise to dramatic metabolic differences between the nontransgenic and transgenic animals (Table 1). As in the previous experiment, stimulation of liver or spleen SSAT activity in the nontransgenic animals in response to the treatment was not very impressive (1.5-fold in the liver, 2-fold in the spleen). Nonetheless, the treatment resulted in an obvious activation of polyamine catabolism, as indicated by the accumulation of putrescine and *N*¹-acetylspermidine, and in decreases in the higher polyamine pools. A compensatory increase in ODC activity after the treatment was also obvious despite high tissue levels of analog. The analog seemed to have a dramatic effect on tissue polyamine metabolism in the transgenic animals. SSAT activity increased by a more than 800-fold in liver and nearly 40-fold in spleen. This was accompanied by almost complete disappearance in the liver of spermidine and spermine and a further increase in putrescine. Spermidine and spermine pools in the spleen were reduced to a lesser extent (Table 1). Because of the huge increase in liver SSAT activity, we again performed a Northern blot analysis of liver SSAT RNA. Unlike with the 24-h treatment, where there were only minor effects on the SSAT message (Fig. 1), the longer treatment resulted in a consistent 4-fold increase in the SSAT mRNA as shown in Fig. 2. This increase was limited to the mature message, as the larger transcript representing preprocessed hnRNA (Fogel-Petrovic et al. 1996) was relatively unaffected. This steady-state level of SSAT mRNA was more than 70 times higher than that seen in the nontransgenic littermates. Despite this difference in mRNA, the level of SSAT activity in nontreated transgenic animals was only 2-fold higher than in treated or untreated nontransgenic mice, suggesting a major role for translational regulation of SSAT gene expression.

As shown in Table 2, brain appeared to be only marginally affected by DENSPM. The 4-fold increase in brain SSAT activity was accompanied by an increase in putrescine, but the higher polyamines, spermidine and spermine, were unchanged (Table 2). This relatively minor effect was apparently due to the low levels of analog accumulated in the brain

(Table 2). The changes of the polyamine pools in small intestine in drug-exposed transgenic animals were very similar to those found in liver and spleen (Table 2). Because skin is clearly affected by overexpression of SSAT (i.e., hairless phenotype), we also analyzed the skin polyamines in DENSPM-treated (4 days) and untreated transgenic mice. As indicated in Table 2, analog treatment typically lowered skin spermidine and spermine pools and caused additional accumulation of putrescine.

Because the above experiments suggested a greater sensitivity of transgenic animals to analog treatment, toxicity studies based on survival were carried out. Nontransgenic (*n* = 15) and transgenic (*n* = 16) animals were treated daily by i.p. injection with 125 mg/kg DENSPM. As shown in Fig. 3, all the transgenic animals died by day 7 (average day of death 5.3 ± 0.9), while all of the nontransgenic mice died by day 10 (average day of death 8.3 ± 0.9). By statistical analysis, this difference was found to be highly significant (*p* < .001).

Discussion

Transgenic animals have typically been used to probe gene function in tissue development and oncogenesis. As shown in this study, they can also be useful in defining the mode of drug action. The exaggerated responses seen in transgenic animals displaying a tissue-wide overexpression of the SSAT gene provides a system for identifying drugs that either inhibit or activate polyamine catabolism and for defining the metabolic and biologic consequences of this drug action. This especially applies to the polyamine analogs, such as DENSPM, where mounting experimental evidence indicates that induction of SSAT seems to represent a major determinant of antitumor drug action in vivo (Bernacki et al. 1995). Thus, although analog suppression of polyamine biosynthesis may contribute passively to polyamine depletion, it would seem that, by increasing polyamine catabolism, SSAT plays the more active role. The rapid depletion of intracellular polyamine pools may also allow higher concentrations of the analog to accumulate in tissues. As seen here, SSAT was potently induced, while the characteristic down-regulation of key biosynthetic enzyme activities, a response once thought to be critical to drug action (Porter and Bergeron, 1988), was not observed. Instead, ODC activity was actually up-regu-

TABLE 2
Effect of DENSPM SSAT activity and polyamine pools in nontransgenic and transgenic animals^a

Tissue/treatment	SSAT Activity (<i>pmol</i> /mg protein/min)	Polyamine pools (<i>pmol</i> /mg tissue wet weight)				
		(<i>pmol</i> /mg tissue wet weight)				DENSPM
		Put	Ac-Spd	Spd	Spm	
Brain (tg-)	11 ± 1	<5	<5	274 ± 38	240 ± 10	
Brain (tg-) + DENSPM	11 ± 3	16 ± 5	<5	329 ± 61	220 ± 17	13 ± 4
Brain (tg+)	18 ± 4	173 ± 93*	13 ± 5*	219 ± 23	311 ± 28*	
Brain (tg+) + DENSPM	74 (95,53)	285 (323,246)	17 (19,15)	189 (208,169)	285 (264,306)	19 (20,18)
Intestine (tg-)	21 ± 5	33 ± 9	<5	1031 ± 183	583 ± 126	
Intestine (tg-) + DENSPM	33 ± 35	99 ± 29	<5	934 ± 179	490 ± 90	230 ± 74
Intestine (tg+)	24 ± 16	1095 ± 332**	42 ± 3***	800 ± 186	709 ± 112	
Intestine (tg+) + DENSPM	593 (686,499)	1474 (1475,1473)	45 (48,41)	86 (88,83)	172 (177,167)	841 (915,767)
Skin (tg+)	n.d.	413 ± 131	40 ± 4	372 ± 27	237 ± 29	
Skin (tg+) + DENSPM	n.d.	1000 (939,1060)	89 (74,104)	100 (64,136)	78 (47,109)	348 (367,329)

^a For experimental details see Table 1. In skin, the statistical significances refer to the treatment effect. n.d., not determined.

lated, presumably in response to analog depletion of the natural polyamines spermidine and spermine via SSAT induction. Why ODC was not down-regulated by the massive amounts of analog present in the tissues may be because the analog is somehow sequestered intracellularly and only able to interact at regulatory sites involved in SSAT induction. Sequestration might also account for the unexpectedly higher levels of analog seen in transgenic tissues, although this could also be at least partially due to depletion of spermidine and spermine pools.

It was also obvious that analog-mediated induction of SSAT could enhance host toxicity. As has been reported (Casero et al. 1989; Porter et al. 1991; Shappell et al. 1992; Casero et al. 1992), cell line cytotoxicity by polyamine analogs in vitro appears to be closely linked to induction of SSAT activity that is known to facilitate activation of polyamine catabolism and/or excretion and the subsequent depletion of spermidine and spermine pools (Seiler, 1987). Whether oxidation of the acetylated polyamines by polyamine oxidase is also related to the cytotoxicity through oxidative stress is undetermined at the present time and may, in fact, be cell-type dependent. In agreement with in vitro studies (Casero et al. 1989; Porter et al. 1991; Shappell et al. 1992), transgenic mice with highly inducible SSAT overexpression appear to be more sensitive to the toxic action of the polyamine analog, DENSPM. The modest induction of SSAT in tissues of nontransgenic animals is in accordance with earlier in vivo studies (Bernacki et al. 1995). Although DENSPM is one of the most effective and best tolerated compounds of the polyamine analog category (Bernacki et al. 1995), the drug caused a substantial mortality to the transgenic animals at dose levels that were well below those that are well tolerated by normal tumor-bearing mice (Bernacki et al. 1995). A critical consideration that still needs to be defined is whether the observed host toxicity is related to antiproliferative effects as in cell culture or to various other physiological responses mediated directly by the analog or indirectly by polyamine depletion. In this regard it is significant to note that, with the exception of the brain, all transgenic tissues accumulated at least 2-fold more DENSPM than nontransgenic tissues. Thus, the in-

creased sensitivity of transgenic animals may be related to more efficient tissue accumulation of the analog, which in turn could bring about greater depletion of spermidine and/or spermine pools due to greater SSAT expression. It should be noted, however, that fibroblasts isolated from transgenic fetuses are much more sensitive to growth inhibition by DENSPM than those from nontransgenic fetuses under conditions where there is no significant difference in the amount of intracellular analog (Alhonen et al. 1998). This apparent paradox could reflect basic differences between the role of SSAT in antiproliferative effects versus whole organ toxicities.

Earlier findings have indicated that substantial increases in SSAT message achieved by transfection or drugs is only poorly translated in the absence of exogenous polyamines (Fogel-Petrovic et al. 1996) or analogs (Parry et al. 1995a). The induction of mammalian SSAT expression is an extremely complex regulatory process that is known to include transcriptional activation, stabilization of message, enhanced translation, and prolongation of the enzyme protein half-life (Libby et al. 1989; Casero et al. 1990; Porter et al. 1992; Fogel-Petrovic et al. 1993; Parry et al. 1995a; Coleman et al. 1995; Fogel-Petrovic et al. 1996, 1997). This multilevel enhancement of gene expression by DENSPM results in a remarkable increase in enzyme activity in transgenic animals that far exceeds the difference in gene copy number. Because similar findings were obtained with metallothionein-driven SSAT transgenic mice (S. Suppola, M. Pietilä, L. Alhonen, J. Jänne, unpublished data), the integration site is not considered to be a factor.

The situation in the SSAT transgenic animals very much resembles the findings obtained with cycloheximide treated (Fogel-Petrovic et al. 1996) or transiently transfected cells (Parry et al. 1995a). The amount of SSAT-derived mRNA in the liver of transgenic animals was nearly 70 times higher than that found in the nontransgenic litter-mate (Fig. 2), yet the actual enzyme activity was only marginally more than that seen in the untreated transgenic liver (Table 2). Apparently, the large amounts of SSAT message produced in transgenic tissues are not readily translated, a process that seems to be activated by exposure to exogenous polyamines (Fogel-Petrovic et al. 1996) or, more efficiently, by analogs as shown here and elsewhere (Fogel-Petrovic et al. 1996; Parry et al. 1995a). The participation of analogs in this event is not straightforward and appears, from the present study, to involve some dose or time factor. A single injection of DENSPM produced an analog level in the liver that was only 30 to 50% lower than that obtained after multiple administrations. This level increased SSAT activity by approximately 10-fold and had little effect on the mRNA level. By contrast, multiple injections increased analog level by 2-fold, raised SSAT message level by about 4-fold, and massively enhanced enzyme activity by approximately 800-fold. This would suggest that processes related to message and protein accumulation may require threshold levels of some additional component, possibly the SSAT protein itself.

The remarkable activation of SSAT gene expression obtained in response to DENSPM in the absence of down-regulation of biosynthetic enzymes provides the first definitive indication of the means by which this clinically-relevant analog depletes tissue polyamine pools in vivo. The data further emphasize the central role of SSAT in maintaining

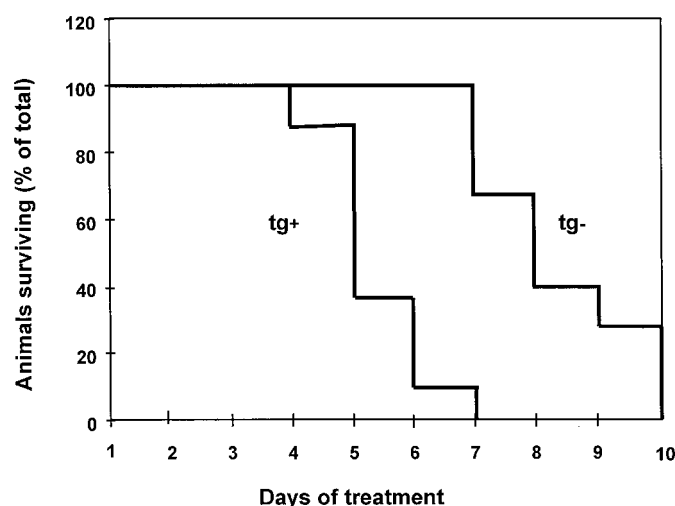


Fig. 3. Effect of DENSPM (125 mg/kg daily) on the survival of nontransgenic ($n = 15$) and transgenic ($n = 16$) mice (Tg-, nontransgenic; Tg+, transgenic). Note that the transgenic mice are significantly ($p < 0.001$) more sensitive to DENSPM treatment.

the homeostasis of the higher polyamines. Thus, these interesting mice will undoubtedly prove useful in identifying second generation analogs with reduced host toxicity and in determining the functional role of polyamines in the context of the whole animal.

Acknowledgments

We thank John Miller and Paula Diegelman for their skilled technical assistance. We also thank Barbara Holdridge for taking care of animal breeding.

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