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Toxic Effects of Secalonic Acid D in Mice and Protection by Dimethylsulfoxide

M. M. R. Eldeib and C. S. Reddy

Department of Veterinary Biomedical Sciences, University of Missouri-Columbia, Columbia, Missouri 65211, USA

Secalonic acid D (SAD) is an acutely toxic (Reddy, et al. 1979), and teratogenic (Reddy et al. 1981) mycotoxin capable of inducing respiratory lesions following intratracheal instillation (Sorenson et al. 1982) and increased mortality in mice subjected to influenza virus infection (Fleischacker et al. 1986). Public health significance of SAD may relate to increasing respiratory disease among occupations dealing with corn harvesting (farmers), storage (grain elevators), and mixing of corn-based feeds. Recent evidence from our laboratory (Eldeib and Reddy 1989) demonstrated that exposure to SAD in both male and female mice results in increased plasma corticosterone levels. Glucocorticoids are known to alter blood cell populations (Haynes and Murad 1985). SAD, in addition, has been shown to cause pulmonary, cardiac and surface liver lesions (Reddy et al. 1979). Organ damage is associated with increases in circulating enzymes. Dimethylsulfoxide (DMSO) has been shown to protect against the teratogenic (Eldeib and Reddy 1988) but not against the acute lethal effects of SAD in males (Reddy et al. 1979) suggesting a complexity of interaction between SAD and DMSO. Since the toxicity of SAD on blood cells, chemistry and enzyme parameters have not been studied, this study was undertaken to evaluate such effects in treated males and to assess the possible prevention of these effects by DMSO.

MATERIALS AND METHODS

Adult (8-9 week old) male CD1 mice from Charles River (Wilmington, MA) were treated, intraperitoneally, with SAD either in 5% $NaHCO_3$ or 20% DMSO in 5% $NaHCO_3$ (v/v) at 0, 15, 30 or 45 mg/kg doses. Water and food consumption and urine and fecal excretion were quantitated by placing groups of 3 mice in metabolism cages. Body temperature was measured using a rectal thermometer (Will Ross, USA). Forty eight hours following dosing, animals were weighed, anesthetized with pentobarbital and blood was drawn by cardiac puncture using heparinized syringes. Liver and Kidneys were excised, weighed and a portion fixed in buffered formalin for routine H & E staining. Freshly drawn blood was subjected to routine hematological analysis including packed cell volume, total white cell count, differential count, red cell count, hemoglobin (Hb), Hb concentration, mean corpuscular (MC) volume, MC hemoglobin (MCH) and MCH concentration (MCHC). Counting was performed using an automated blood cell counter (Coulter Electronics Inc., Hialeah, FA). Plasma was used for total protein determination using Model 10400 ATS (American Optical Instrument Co., Buffalo, NY) refractometer, (Silverman,

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Address correspondence to Dr. Reddy at the above address

et. al., 1986). Plasma protein separation and quantitation were performed using cellulose acetate electrophoresis and simultaneous densitometric scanning (Helena Laboratories, Beaumont, TX). Pooled urine samples from groups of 3 mice were centrifuged, lyophilized and reconstituted in 0.5 ml of deionized water before electrophoresis. Urinary protein was determined by the method of Bradford (1976).

Plasma enzymes were assayed in the clinical pathology laboratory using the automated system of American Monitor Corporation (Indianapolis IN) based on the following methods. Lactate dehydrogenase (LDH) was proportional to the intensity of the blue color (610 nm) generated by the formation of Fe^{+3}-AM blue complex following reduction of Fe^{+3} to Fe^{+2} by the NADH generated as a result of LDH action on lactate in the presence of NAD (McComb 1983). Alkaline Phosphatase (ALP) was determined by quantitating the yellow color (410 nm) due to p-nitrophenol formed from the hydrolysis of p-nitrophenylphosphate by the action of ALP (Teitz and Rinker 1983). Glutamate - Oxaloacetate transaminase (GOT) activity was determined by measuring the decrease in absorbance at 340 nm, reflecting a loss of NADH in the reaction catalyzed by malatedehydrogenase involving conversion of oxaloacetate to malate. Oxaloacetate was generated in the transamination reaction involving L-aspartate and α-ketoglutarate catalyzed by GOT (Moss et. al. 1986). Creatine kinase (CK) was determined manually using a kit from Scalvo (Florentina, Italy) based on the conversion of ADP to ATP, the phosphate being derived from the conversion of creatine phosphate to creatine by the action of CK. ATP is then used in the conversion of glucose to glucose-6-phosphate (G6P) by hexokinase and finally G6P is converted to 6-phosphogluconate with simultaneous formation of NADPH from NADP. The decrease in absorbance of NADP was considered proportional to CK activity (Meiattini, et al. 1978). All enzyme activities were expressed as units/liter (U/L). A unit (U) is defined as the amount of enzyme capable of catalyzing one micromole of a given substrate per minute.

The effect of dose on various parameters was evaluated using the analysis of variance followed by Duncan's multiple range test. Comparison of means at each dose between $NaHCO_3$ and DMSO was done using Students' t-test. A P value of $<.05$ was considered significant.

RESULTS AND DISCUSSION

Animals receiving 45mg/kg SAD in $NaHCO_3$ had lower body and liver weights whereas those receiving the same dose in DMSO had higher body weight and similar liver weight compared to their respective controls (Table 1). DMSO itself caused a significant increase in liver to body weight ratio (Table 1) compared to $NaHCO_3$ controls. Rectal temperature was significantly lowered in mice receiving 45mg/kg of SAD in $NaHCO_3$, which was only partially prevented by DMSO. Other parameters that were reduced by SAD without being prevented by DMSO, include quantitative fecal excretion, water intake and urine volume (not shown). Given in $NaHCO_3$, all doses of SAD produced significant increases in segmented neutrophils (Segs) and total WBC (Fig 1). DMSO, by itself, significantly increased both Segs and WBC but did not alter lymphocyte number compared to $NaHCO_3$ controls. Given in DMSO, only the highest dose of SAD (in DMSO) produced a significant increase in Segs and total WBC suggesting some protection by DMSO. All doses of SAD, whether given in $NaHCO_3$ or in DMSO, significantly reduced lymphocyte numbers compared to

Table 1. Effect of secalonic acid D in $NaHCO_3$ or DMSO on organ/body weight ratios, urinary protein excretion, and body temperature in mice, 48 hours following administration.

Parameter	Dose of SAD (mg/kg) in $NaHCO_3$		DMSO	
	0	45	0	45
Body Weight(g) gain(+) or loss(-)	+0.29 ± 0.13	-1.39[a] ± 0.67	-0.19 ± 0.16	0.57[b] ± 0.63
Liver Weight(g)	1.92 ± 0.14	1.54[a] ± 0.08	1.95 ± 0.19	1.95[b] ± 0.34
Liver Weight X 100 / Body Weight	4.8 ± 0.2	4.8 ± 0.1	5.9[b] ± 0.4	5.3 ± 1.1
Total Urinary Protein[c] (mg/48 hr)	15.71	5.32	---	4.54
Body Temperature (Rectal,°F)	102.5 ± 0.2	99.7[a] ± 0.4	---	100.7 ± 0.1

[a] Significantly ($P<0.05$) different from the respective controls.
[b] Significantly ($P<0.05$) different from the same dose in $NaHCO_3$.
[c] Value obtained from a urine pool of 3 mice housed together in single metabolism cage.

controls. DMSO itself failed to alter lymphocyte numbers. There were no significant differences at any of the SAD doses (15-45 mg/kg) between $NaHCO_3$ or DMSO groups of the parameters examined (Fig. 1).

In the absence of DMSO, 15 to 45 mg/kg of SAD increased total WBC to between 115 and 220% and segmented neutrophils to between 290 and 400% of controls. Presence of DMSO reduced this response to between 90 and 140% and 140 and 190% of controls, respectively. Lymphocyte count was reduced to 55% of that of controls irrespective of the presence of DMSO. Glucocorticoids have been shown to reduce recruitment of circulating neutrophils to the sites of inflammation, reduce the sensitivity of lymphocytes to mitogens (Haynes and Murad 1985) and produce lymphocytolysis by facilitating programmed cell death mediated by the glucocorticoid receptor which in turn may either induce a gene product with lytic function or repress essential gene products (Yamamoto 1985). The demonstration of increased circulating corticosterone levels by SAD in male CD1 mice (Eldeib and Reddy 1989) lends support to the role of corticosterone in SAD-induced blood cell changes. DMSO-induced cellular changes in our study appear not to be associated with a rise in plasma corticosterone levels (Eldeib and Reddy, 1989) suggesting different mechanism of action for DMSO. Neither SAD nor DMSO altered the red cell count or red cell parameters examined.

Plasma albumin and α_1 globulin were reduced by SAD only at the highest dose given in either solvent (Figs. 2 and 3). The response, however, was significantly greater in mice receiving SAD in $NaHCO_3$. The lack of effect on gamma globulins in spite of lower number of

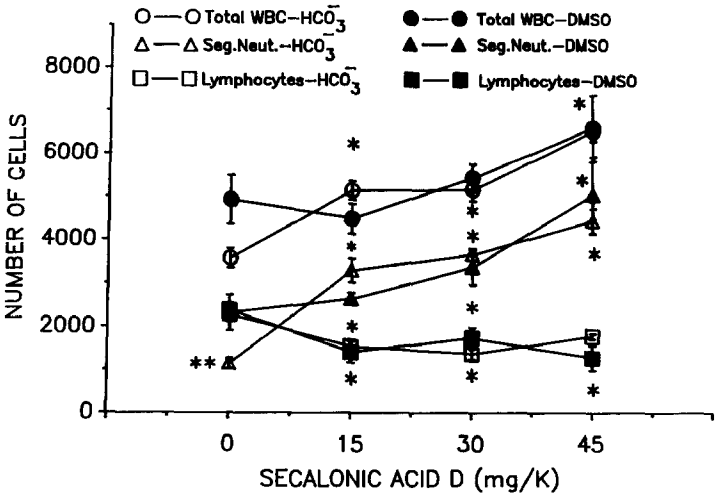

Figure 1. Effect of Secalonic Acid D on circulating white blood cells in mice. Asterisks represent significant difference ($P<.05$) from respective control (*) or from the DMSO group (**).

circulating lymphocytes suggests that this effect may be related to redistribution rather than actual reduction in number, an action similar to that of glucocorticoids (Haynes and Murad 1985). Alternatively, it may indicate a specific effect on T-lymphocytes. Studies to evaluate the role of proteinuria in the production of hypoproteinemia indicated that SAD-treated animals actually excreted less protein than $NaHCO_3$ controls and that the major protein excreted was electrophoretically different from albumin (Table 1, and Fig. 4). This, together with absence of kidney or liver lesions in SAD-treated mice (present study and Reddy et al. 1979) suggests that hypoproteinemia may be due to SAD-induced anorexia and subsequent starvation and not due to organ damage. DMSO, although partially corrected SAD-induced hypoproteinemia, failed to reverse specific decreases in albumin and α_1-globulin levels.

SAD significantly reduced plasma ALP activity in mice at only 15 mg/kg in $NaHCO_3$ but required 30 mg/kg when given in DMSO (Fig-5). Given in $NaHCO_3$, SAD significantly increased plasma LDH, GOT and CPK activities but only at higher dose levels (30 and/or 45 mg/kg). DMSO itself significantly increased the activities of these enzymes (Table 2). These enzyme activities in animals given SAD in DMSO were for the most part similar to those of SAD given in $NaHCO_3$. Expressed as a percent of respective control, however, it is evident that SAD given in $NaHCO_3$ increased the activities of all enzymes except ALP whereas SAD given in DMSO reduced the activity of all enzymes studied (Fig. 5). Decrease in ALP activity as produced by SAD in this study may be secondary to increase in corticosterone levels (Bijlsma et al. 1988) by SAD. Requirement of a higher dose

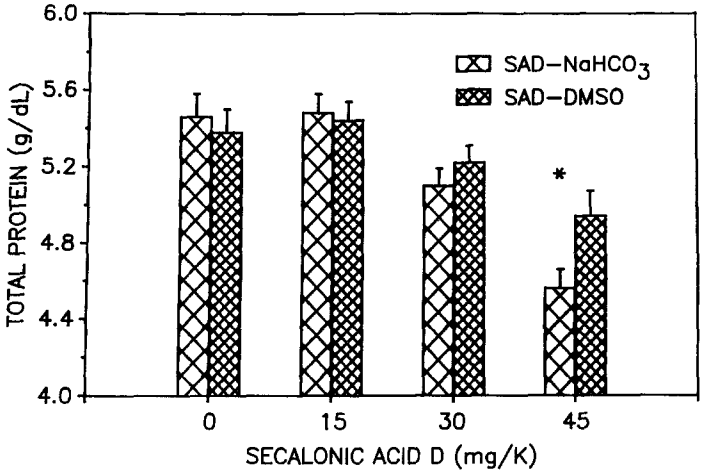

Figure 2. Effect of Secalonic Acid D on Plasma Protein in Mice. (*) Significantly (P<·05) different from $NaHCO_3$ and 45 mg/kg SAD in DMSO

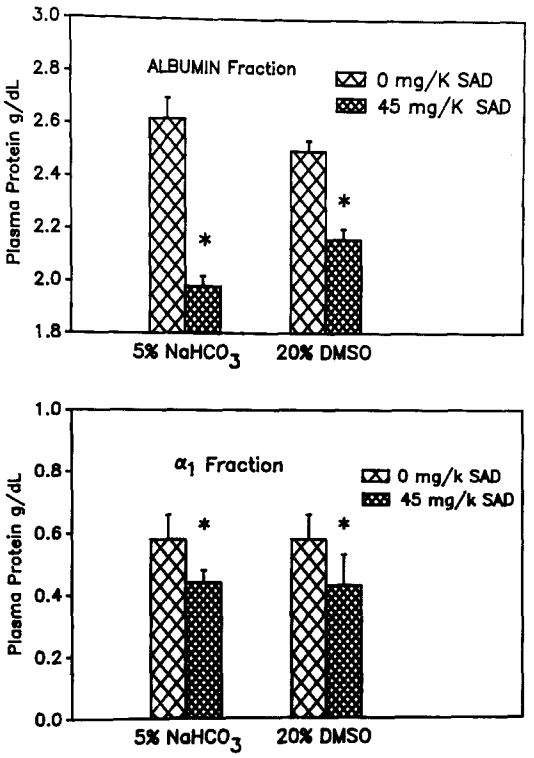

Figure 3. Plasma Albumin and α_1, Globulin levels in Mice as Affected by Secalonic Acid D. (*) Significantly (P<·05) different from its control (t-test).

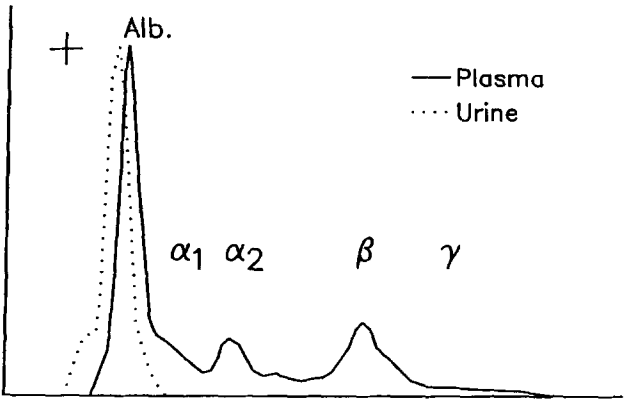

Figure 4. Plasma and Urine Electrophoresis Profile. Alb. α_1, α_2, β and γ represent albumin and globulin fractions after SAD treatment respectively.

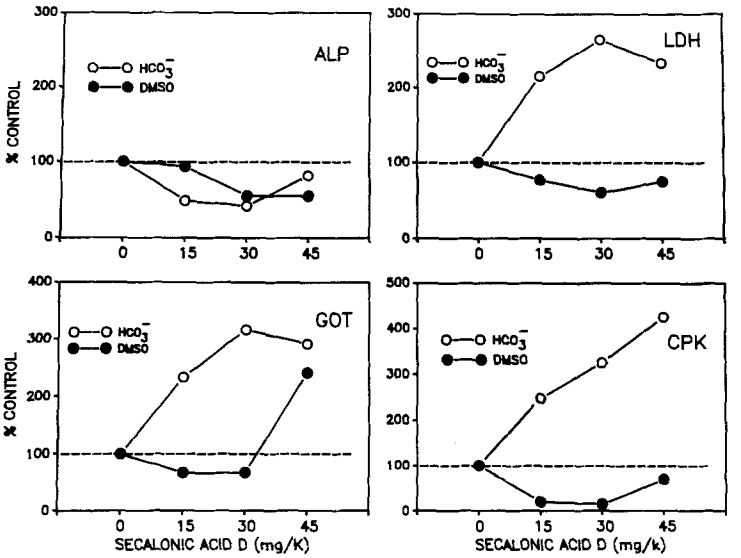

Figure 5. Effect of Secalonic Acid D on Plasma Enzymes in Mice.

of SAD in the presence of DMSO to induce a comparable decrease in ALP activity (this study) together with DMSO-induced suppression of corticosterone elevation by SAD (Eldeib and Reddy 1989) supports this contention. SAD-induced increases in CPK, LDH and GOT all of which originate predominantly from the heart, may reflect cardiac

Table 2. Plasma Enzyme Levels 48 Hours Following Treatment With Sodium Bicarbonate or DMSO.

Treatment	ALP		LDH		sGOT		CPK	
5% NaHCO3	160	15	213	12[a]	51	2[a]	26	4[a]
20% DMSO	150	22	356	30	124	20	122	21

a Significantly ($P<0.05$) different from the DMSO group.

damage (Reddy et al. 1979) and are unrelated to SAD-induced starvation, as starvation would have produced a decrease in the activity of these enzymes (Mitruka and Rawnsley 1981). DMSO-induced increases in all of these enzymes in our study (Table 2) are likely due to increase in cell membrane permeability possibly mediated by reduced tissue oxygen tension (Ashwood - Smith 1971). In contrast to either DMSO or SAD, the combination produced a decrease in the activities of GOT, CPK and LDH. This suggests the formation of a SAD-DMSO complex in vivo with relatively greater binding to macromolecules (including enzymes) compared to SAD or DMSO alone (Levine 1975).

The results of these studies demonstrated that the toxicosis with SAD is characterized by increases in circulating neutrophils, total WBC, and serum enzymes including CPK, GOT and LDH; and decreases in body temperature, lymphocytes, liver/body weight ratio, fecal weight, urinary volume, total serum protein, plasma albumin and α_1 globulin, urinary protein, and plasma ALP. The protective agent, DMSO, prevented SAD-induced reduction in liver/body weight ratio; only partially prevented SAD-induced decrease in body temperature, total serum protein, and plasma ALP; and failed to prevent decreases in lymphocytes, fecal weights, urine volume, plasma albumin and α_1 globuliln. DMSO itself produced increases in neutrophils, total WBC, and increases in CPK, GOT and LDH and either reduced SAD-induced increases (neutrophils and WBC) or failed to alter SAD-induced changes in these parameters compared to $NaHCO_3$.

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