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Reduced Activities of Serum Lactate Dehydrogenase and Aminotransferases Due to an Oral Administration of 2-Chloroethyl Linoleate in Rats

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Earlier studies from this laboratory have shown in vivo, as well as in vitro formation of fatty acid esters of 2-chloroethanol (Kaphalia and Ansari, 1989; Bhat and Ansari, 1990). Besides their formation in vivo, these esters may also be consumed in diet. 2-Chloroethyl esters of capric (C_{10}), lauric (C_{12}), myristic (C_{14}), palmitic (C_{16}), oleic ($C_{18:1}$), and linoleic ($C_{18:2}$) acids, which are formed as a result of fumigation with ethylene oxide, have been identified in black walnut, spices, fat, oil, and food samples using gas chromatography/mass spectrometry (Heikes and Griffitt, 1979; 1979a; Yurawecz, 1987). Among all the organic residues determined under Total Diet Studies conducted by US Food and Drug Administration, the fatty acid esters of 2-chloroethanol were shown to be present in the highest concentration, of which 2-chloroethyl linoleate (2-CEL) was the major fatty acid ester. The total dietary intake (μg/kg body weight/d) of 2-CEL has been estimated to be 0.079-0.228 for adults and 0.145-0.418 for toddlers (Gartrell et al., 1986, 1986a). To the best of our knowledge, no information is available on toxicity of orally ingested 2-CEL in humans or in experimental animals. Therefore, the present study was undertaken to evaluate the toxicity of 2-CEL in male Sprague-Dawley rats.

MATERIALS AND METHODS

2-CEL was synthesized and characterized according to the procedures described earlier (Kaphalia and Ansari, 1989; Bhat and Ansari, 1990). Male Sprague-Dawley rats (~170 g) obtained from Harlan, Sprague-Dawley Inc., Indianapolis, were acclimated for 7 d in a 12 hr light/dark cycle. The animals were fed Purina Chow diet and tap drinking water ad libitum. Fifteen rats were given 250 mg/kg body weight of 2-CEL in 0.5 ml mineral oil, by gavage. Control rats (twelve) received an equal volume of mineral oil only. Five rats from experimental and four from control groups were sacrificed on each day 2, 4, and 8 following the treatment. Blood was withdrawn from inferior vena cava. A small portion of the whole blood was mixed with an anticoagulant and used for the analysis of hematological parameters on a Coulter Model

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ZB-1 blood cell counter (Coulter Electronics, Inc., Hialeah, FL). The remaining blood was used to obtain the serum. Major organs were excised, washed with saline, blotted and weighed. A portion of the major organs and tissues was fixed in 10% buffered formaldehyde solution and subsequently processed for histopathological examination. Serum and liver cytosolic lactate dehydrogenase (LDH), and L-aspartate:2-oxoglutarate aminotransferase (AST/GOT) and L-alanine: 2-oxoglutarate aminotransferase (ALT/GPT) were determined according to Wroblewski and LaDue (1955) and by using Sigma Diagnostic Kits (Procedure no. 505), respectively.

RESULTS AND DISCUSSION

No significant changes were observed in WBC, RBC and differential nucleated cell populations, hemoglobin contents, mean capsular volume, and platelet counts. However, statistically significant reduction in the serum LDH, AST and ALT activities of 2-CEL-treated rats was observed at most of the time points studied (Table 1). The serum LDH activity was about 54, 43 and 43% of the control values at day 2, 4, and 8, respectively, following the 2-CEL treatment. The decreases in the activities of serum AST and ALT in 2-CEL-treated animals were also significant but less pronounced as compared to the decrease observed for the serum LDH activity. There were no significant changes in the activities of liver cytosolic LDH and aminotransferases of 2-CEL-treated animals as compared to the controls. No histopathological changes were noticed in liver, lung, heart, kidney, testes, intestine, brain, salivary gland and pancreas.

The activities of LDH and aminotransferases are present in almost all cells of the body and are invariably found in the cytoplasm of the cell. Increased serum levels of these enzymes have been reported in a variety of hepatic diseases (including chemically induced) and tissue injuries, such as myocardial infarction (Moss et al., 1986), and thus serve as markers of tissue injury. However, decreased serum activities of these enzymes have rarely been reported. Synthetic fatty acid anilides have been shown to reduce the activities of NADPH-diaphorase of liver post mitochondrial fraction and glucose-6-phosphatase, as well as ATPase in the membrane fraction of rat liver and lung (Sanz et al., 1983; 1986). Recent, studies with linoleic acid anilide (Khan et al., 1991) and 2-chloroethanol (Kaphalia et al., 1991) have also shown a significant decrease in the serum levels of LDH and amino-transferases as a result of their oral administration in rats. The significance and mechanism(s) of such decrease in the activities of serum LDH and amino-transferases are not known. The fatty acid anilides, 2-chloroethanol and 2-CEL, being lipophilic, may have a profound effect on the plasma membrane by causing perturbation in various transport processes. The changes in the membrane properties due to 2-CEL may have an effect similar to that of promethazine which also lowers the secretion of LDH from rat hepatocytes (Dianzani, 1984). The liver cytosolic LDH and aminotransferases were not significantly altered in 2-CEL-treated animals as compared to the

Table 1. The activities of LDH, AST and ALT in the serum and liver cytosol of control and 2-CEL-treated rats.

	Serum (units/ml)			Liver Cytosol (units/mg protein)		
	Day 2	4	8	Day 2	4	8
LDH						
Control	1361 ± 213	808 ± 46	1027 ± 51	2592 ± 232	2473 ± 195	2399 ± 203
Experimental	737 ± 209*	347 ± 33**	438 ± 76***	2102 ± 163	2882 ± 206	2826 ± 349
	(54)	(43)	(43)	(81)	(116)	(118)
AST[a]						
Control	98 ± 7	88 ± 3	107 ± 3	449 ± 26	454 ± 15	494 ± 17
Experimental	80 ± 5	65 ± 3***	79 ± 4***	395 ± 16	454 ± 12	449 ± 13
	(82)	(74)	(74)	(88)	(100)	(91)
ALT[a]						
Control	24 ± 2	20 ± 1	23 ± 2	232 ± 27	221 ± 18	346 ± 53
Experimental	16 ± 1*	15 ± 1*	23 ± 21	90 ± 10	226 ± 14	274 ± 9
	(67)	(75)	(100)	(82)	(102)	(75)

Values are mean ± SE of five experimental and four control animals.
[a] The activities of AST and ALT are expressed in Sigma-Frankel units.
Digits in parentheses are the percent of control.
P values * ≤ 0.05, ** ≤ 0.01, and *** ≤ 0.001.

controls, therefore, necessitates their measurements in other tissues and further studies to understand the mechanism(s) responsible for such changes.

Chronic exposure of 2-CEL to humans through diet should be considered important from a health point of view, since an appreciable amount of 2-CEL is consumed in the diet by the general population in the United States (Gartrell et al., 1986; 1986a). 2-CEL, being lipophilic, may be retained in the body for a long time and accumulate to a sublethal concentration upon chronic exposure. The information regarding the residue levels of 2-CEL in human population and related animal toxicity data is not available. In this preliminary study, we have shown that serum enzymes are affected by 2-CEL. Therefore, this study will be extended in order to investigate the mechanism(s) by which 2-CEL causes decrease in the serum enzymes and its dose-dependent response, no effect level and chronic toxicity in animal model.

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