

Ascorbic Acid and Elevated SGOT Levels after an Acute Dose of Ethanol in the Guinea Pig

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Male guinea pigs were maintained on a vitamin C-deficient chow diet and supplemented with either 0.05 or 2.0 mg of ascorbic acid/ml drinking water for 3 weeks prior to receiving an intraperitoneal injection of 4.0 g of ethanol/kg body weight. The following biochemical parameters were measured prior to, and hourly for 12 hours after, ethanol administration: serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvate transaminase (SGPT), serum triglycerides, and blood ethanol clearance. The animals were killed 12 hours after ethanol administration and liver weight to body weight ratios and hepatic ascorbic acid concentrations determined. Acute ethanol administration resulted in a 12-fold increase in SGOT levels in animals with hepatic ascorbic acid concentrations at or below 16 mg/100 g of liver. A marked reduction, 60%, in this increase was observed in animals that had concentrations of hepatic ascorbic acid above 16 mg/100 g of liver. No effect of hepatic ascorbic acid concentration was observed on elevated levels of SGPT, serum triglycerides, or blood ethanol clearance.

THE ABILITY of ascorbic acid to protect against the toxicity of ethanol has been studied to a limited extent. Yunice and Lindeman¹ found that pretreatment with ascorbic acid protected against the lethality of an acute dose of ethanol in mice and against the lethality of chronic ethanol exposure in the rat. Susick et al.² have demonstrated in the guinea pig that high dietary levels of ascorbic acid protect against hepatic steatosis, necrosis, and elevated levels of serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvate transaminase (SGPT) caused by chronic ethanol consumption. Protection against hepatic steatosis by ascorbic acid was also found by Yunice et al.³ in guinea pigs chronically infused with ethanol. In addition, ascorbic acid has been shown to reverse the impaired swimming behavior of mice treated with an intoxicating dose of ethanol⁴ and to prevent the marked depletion of circulating eosinophils in the rat caused by alcohol.⁵

This acute study examines, for the first time, the influence of ascorbic acid on elevated levels of the serum enzymes glutamic-oxaloacetic transaminase (SGOT) and

glutamic-pyruvate transaminase (SGPT), both indices of increased permeability and/or necrosis of hepatocytes; elevated levels of serum triglycerides, an index of altered fat metabolism; blood ethanol clearance; and liver weight:body weight ratios, after a single dose of ethanol in the guinea pig. The serum enzymes, SGOT and SGPT, were chosen as indices of cellular damage because they are a generally well accepted measure of such damage and have been demonstrated to be elevated after ethanol administration.⁶⁻⁸ One of the primary toxic consequences of ethanol abuse is the fatty liver caused by altered lipoprotein metabolism. Serum triglycerides were chosen as an index of this altered fat metabolism and have been shown to be increased after an acute dose of ethanol.^{9,10} The guinea pig was chosen as the animal model since, like man, it cannot synthesize vitamin C and its metabolism of ethanol more closely resembles human alcohol metabolism than other animal species.^{11,12}

MATERIALS AND METHODS

Source of Chemicals, Materials, and Guinea Pigs

L-Ascorbic acid was purchased from Sigma Chemical Company, St. Louis, MO. Ninety-five percent ethanol was used to make all ethanol solutions. Materials and methods for the determination of SGOT, SGPT, and serum triglycerides were also obtained from Sigma Chemical Co. All chemicals used were of reagent grade or better. Gas chromatography packing material was Porapak Q purchased from the Anspec Company, Ann Arbor, MI. Ascorbic acid deficient guinea pig chow diet was purchased from Nutritional Biochemicals, Cleveland, OH. Male Hartley guinea pigs were purchased from the Michigan Department of Public Health, Lansing, MI.

Animal Treatment

Male Hartley guinea pigs (225-275 g) were maintained on a vitamin C deficient diet and supplemented with either 0.05 or 2.0 mg of ascorbic acid/ml drinking water for 3 weeks prior to use. The animals that received 2.0 mg of ascorbic acid/ml drinking water had a normal intake of the vitamin as indicated by their rate of weight gain and hepatic concentrations of the vitamin. One hour prior to ethanol administration, animals on the high ascorbic acid diet were given an additional 100 mg of ascorbic acid orally diluted in 2.0 ml of water and the animals on the low ascorbic acid diet were given 2.0 ml of water alone. The ascorbic acid regimens were chosen to provide animals with varying hepatic concentrations of the vitamin. None of the animals on the low ascorbic acid regimen developed joint hemorrhages or suffered any weight loss. Animals were housed two per cage at $22 \pm 2^\circ\text{C}$ on a 12-hr light cycle. Animals were fasted overnight before alcohol administration and weighed from 400-550 g at time of alcohol administration. Blood samples were taken in the morning 2 hr prior to ethanol injection by toenail clipping to establish baseline values for the serum enzymes and serum triglycerides. Ethanol

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was injected intraperitoneally at a dose of 4.0 g/kg as a 30% solution in saline. Prior to the establishment of this dose of alcohol, various dosing regimens were performed. After consideration of the following factors; response, blood ethanol levels, ethanol clearance and survival, the dose was chosen. Blood samples were taken hourly after ethanol injection up to 12 hr by toenail clipping for determination of the serum enzymes, triglycerides, and ethanol concentration. All of the biochemical parameters peaked within 12 hours.

Fifty microliters of blood was used for ethanol determination and was added to 1.0 ml of 5% perchloric acid containing 25 mM thiourea in a 25-ml serum bottle followed by addition of 50 μ l of 5 mM isopropyl alcohol which was used as an internal standard. The bottles were immediately sealed after addition of the internal standard. The remaining blood, approximately 0.25 ml, was centrifuged at $10,000 \times g$ for 5 min and serum collected for determination of SGOT, SGPT, and serum triglycerides. The serum was immediately refrigerated. Studies indicated that these samples were stable at 4°C for at least 1 week. Samples of deproteinized blood for ethanol determination were assayed the following day.

Twelve hours after ethanol injection, the animals were weighed, decapitated, and livers weighed and homogenized in four volumes of cold 0.1 M sodium phosphate buffer, pH 7.4. Hepatic ascorbic acid concentrations were determined by the method of Zannoni et al.¹³

Measurement of SGOT and SGPT

Samples of serum were incubated with either DL-aspartate and α -ketoglutarate for SGOT determination or DL-alanine and α -ketoglutarate for SGPT determination. The oxaloacetic acid or pyruvic acid formed was then reacted with 2,4-dinitrophenylhydrazine to form an adduct which absorbs light at 505 nm. Materials for the determination of SGOT and SGPT were obtained from Sigma Chemical Co.

Measurement of Serum Triglycerides

Triglycerides were measured by enzymatic conversion to pyruvate by utilizing the following enzymes; lipase, glycerokinase, and pyruvate kinase. The pyruvate was then reduced to lactate by lactate dehydrogenase and NADH. The decrease in absorbance at 340 nm is proportional to the amount of triglycerides present. Materials for the determination of serum triglycerides were obtained from Sigma Chemical Co.

Blood Ethanol Clearance

Serum bottles containing the deproteinized blood for ethanol determination were heated at 60°C for 15 min before injection of 5 cc of head space gas into a Varian Model 3700 gas chromatograph equipped with a flame ionization detector. The column dimensions were 2 meters \times 4 mm intradermal and packed with Porapak Q. The column temperature was 180°C, and the detector and injector temperatures were 200°C. The carrier gas was nitrogen with a flow rate of 17 ml/min. Standard curves were plotted as the peak height ratios of ethanol/isopropyl alcohol. Values of blood ethanol concentration were plotted against time and clearance calculated from the slope of the regression line.

Statistical Analysis

Statistical significance of the data was determined by the two-sided Student's *t* test. Differences were considered significant at a *p* value < 0.05.

RESULTS AND DISCUSSION

The correlation between the concentration of hepatic ascorbic acid and the levels of SGOT after the acute dose of ethanol is illustrated in Fig. 1. Animals on the low and

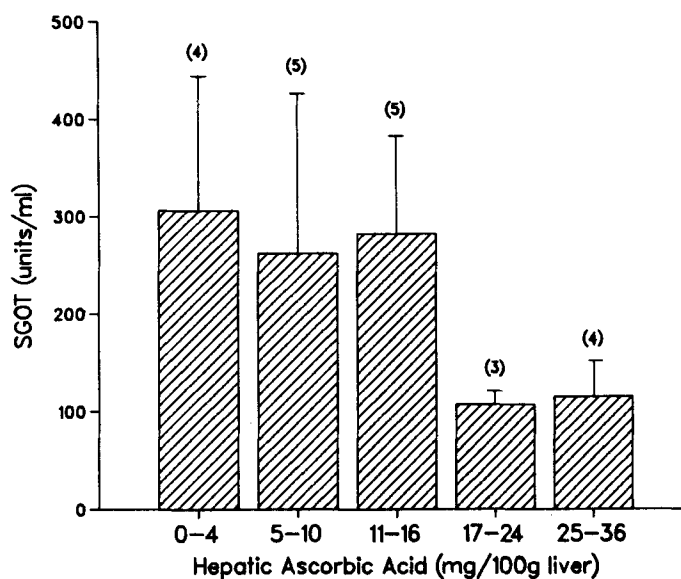


Fig. 1. Correlation of SGOT levels and hepatic ascorbic acid concentration after an acute dose of ethanol. 1 unit = 4.82×10^{-4} μ moles glutamate formed/min at pH 7.5, 25°C. SGOT levels and hepatic ascorbic acid concentrations were determined after an intraperitoneal injection of 4.0 g of ethanol/kg body weight in animals maintained on a low ascorbic acid regimen (these animals had hepatic ascorbic acid concentrations from 0–10 mg/100 g of liver), and in animals maintained on a high ascorbic acid regimen (these animals had hepatic ascorbic acid concentrations from 11–36 mg/100 g of liver). Animals were divided into groups according to their hepatic ascorbic acid concentration. Number in parentheses represents number of animals.

high ascorbic acid regimen are divided into groups according to the quantity of hepatic ascorbic acid. Animals on the low ascorbic acid regimen had hepatic concentrations of the vitamin from 1–10 mg/100 g of liver and experienced a 12-fold increase in SGOT levels after the acute dose of ethanol. Animals on the high ascorbic acid regimen that had hepatic ascorbic acid concentrations somewhat higher, 11–16 mg/100 g of liver, also experienced a 12-fold increase in SGOT levels. In contrast, animals on the high ascorbic acid regimen that had concentrations of hepatic ascorbic acid above 16 mg/100 g of liver experienced only a 5-fold increase. These data suggest a threshold concentration of ascorbic acid below which one is susceptible to enhanced ethanol toxicity resulting in the release of this enzyme into the blood. A statistically significant increase in SGOT levels is observed in animals that have concentrations of hepatic ascorbic acid at or below 16 mg/100 g of liver when compared to animals that have concentrations of the vitamin above 16 mg/100 g of liver, $p < 0.01$ (Table 1).

Ethanol is known to disorder membranes, expanding their area, making them more fluid, and increasing the mobility of membrane components.^{14,15} Ethanol also acts as a direct hepatotoxicant resulting in necrosis.^{15,16} Elevation in SGOT levels is indicative of the increased permeability and/or necrosis of hepatocytes. The ability of high hepatic concentrations of ascorbic acid to reduce the release of this enzyme may be due to its involvement either in membrane stabilization or prevention of the biochemical events leading to increased permeability or necrosis.

Table 1. Effect of Hepatic Ascorbic Acid Concentration on Indices of Acute Ethanol Toxicity

	SGOT (units/ml)*	SGPT (units/ml)	Serum triglycerides (mg/100 ml)	Liver weight
				Body weight (mg/g)
Hepatic ascorbate (mg/100 g liver)				
1-16	281 ± 140 (14)	64 ± 24 (14)	200 ± 86 (12)	46 ± 7 (14)
17-36	111 ± 26 (7)	53 ± 12 (7)	213 ± 101 (7)	40 ± 2 (7)
p value†	<0.01	NS‡	NS	<0.05
Baseline before alcohol	22 ± 9 (21)	17 ± 8 (21)	121 ± 37 (19)	

Values represent means ± SD, with number of animals in parentheses, after 4.0 g of ethanol/kg ip. Baseline values taken 2 hr prior to ethanol administration were not affected by ascorbate concentration and were combined.

* 1 unit = 4.82×10^{-4} μ moles glutamate formed/min.

† p values calculated from a two-sided Student's *t* test.

‡ NS, not significant, *p* > 0.05.

With regard to the possible mechanism of ascorbic acid action, it is known that the metabolism of ethanol via alcohol dehydrogenase causes an increase in the cellular NADH/NAD⁺ ratio which causes a host of metabolic derangements.^{16,17} If these metabolic derangements are severe enough, cell injury and release of enzymes can result. Ascorbic acid may be able to provide an alternate, nontoxic, pathway for ethanol metabolism thereby sparing the increased NADH/NAD⁺ ratio. In keeping with this, we have previously described an ascorbic acid-dependent alcohol oxidizing system that utilizes catalase to oxidize ethanol to acetaldehyde.¹⁸ In addition, ascorbic acid may be acting to stabilize membrane function and transport which is altered by ethanol. Ascorbic acid has been shown to be involved in Na⁺/K⁺ transport¹⁹ as well as amino acid transport across the cornea membrane.²⁰ A role for the vitamin has also been demonstrated in reducing permeability of epithelial cells of mucosal tissue²¹ and in preventing fragility and permeability of the peripheral vascular system.²²

Although there was significant reduction in the elevation of SGOT levels in animals with hepatic concentrations of ascorbic acid above 16 mg/100 g of liver (Fig. 1, Table 1), no correlation was found between concentrations of the vitamin and levels of SGPT or serum triglycerides. It is not unusual that SGOT and SGPT levels do not rise concomitantly in response to a toxicant.²³ The two enzymes are located in different subcellular compartments of the cell and depending on the degree and type of injury, the enzymes may be released in different amounts and at different times. A slight but significant difference did exist however, in liver weight:body weight ratios. Animals with hepatic ascorbic acid concentrations above 16 mg/100 g of liver had a lower liver:body weight ratio than animals with ascorbic acid concentrations at or below this level (Table 1).

Blood ethanol clearance was also compared between the two groups. Previous investigators have found in the guinea pig that pretreatment with ascorbic acid enhances blood ethanol clearance. Yunice et al.³ pretreated carotid-cannulated guinea pigs with an infusion of ascorbic acid prior to ethanol infusion which resulted in a 35% enhancement in blood ethanol clearance. Under our experimental

conditions however, there was no significant enhancement of clearance. The mean rate of ethanol clearance from the blood for animals with 0-16 mg of ascorbic acid/100 g of liver was 773 μ moles/100 ml/hr ± 104 (9) and 755 ± 55 (7) for animals with 17 to 36 mg/100 g of liver.

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