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Influence of Vitamin C Status on Ethanol Metabolism in Guinea-Pigs

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Summary

Guinea-pigs were maintained for 5 weeks on a diet containing three different concentrations of vitamin C: a) traces (none added), b) medium (0.05 % w/w) and high (0.5 % w/w). Twenty-four hours before killing the animals received one i.p. dose of 3 g ethanol per kg body weight (a model of short-term acute intoxication). In a parallel experiment which lasted 5 weeks, the animals were treated every week with two i.p. doses of 1 g ethanol per kg body weight followed by the final acute intoxication (3 g ethanol/kg) (a model of long-term chronic alcoholization). In both experiments, the guinea-pigs with the highest tissue concentration of vitamin C proved to have significantly decreased residual levels of ethanol and acetaldehyde in the liver and the brain, a decreased activity of alanine- and aspartate aminoacyl transferases in the serum and decreased contents of triacylglycerols and cholesterol in the serum and liver in comparison with the vitamin C-unsupplemented group. The regression curve expressing vitamin C levels versus residual ethanol and acetaldehyde concentrations in the liver confirmed the highly significant negative correlation between them. Administration of the guinea-pigs with large amounts of vitamin C appears to accelerate ethanol and acetaldehyde metabolism and reduce some of their adverse health effects.

Key words
Vitamin C – Ethanol – Acetaldehyde – Metabolism

Introduction

Ethanol exerts well-known systemic adverse effects on human health. As an example of the metabolic disturbance related to increased alcohol uptake and enhanced vitamin C catabolism, an elevated vitamin C need in guinea-pigs and man have been suggested (Seitz and Suter 1994). Indeed, impaired vitamin C status in human alcoholics has repeatedly been found (Fazio *et al.* 1981) and enhanced biosynthesis of ascorbate in rats intoxicated by alcohol has been estimated experimentally (Zloch and Ginter 1995). The alteration of vitamin C metabolism associated with alcohol abuse seems to be caused by the active participation of this vitamin in ethanol turnover, especially in some alternative metabolic pathways of this toxicant, the microsomal ethanol oxidizing system being the most important (Song and Cederbaum 1996).

The active function of ascorbate in these processes may be reflected in the dynamics of ethanol oxidation and its elimination from the body. While the stimulating role of vitamin C related to the oxidative biotransformation of other noxious substances and xenobiotics has been well-known for a long time (Zannoni and Lynch 1973), its significance in the ethanol catabolism remains to be clarified. The present contribution was aimed to elucidate the potential role of ascorbic acid in alcohol detoxification.

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Methods

Male guinea-pigs (380 g body weight) fed laboratory chow were used in two parallel experiments in which the animals either received a single-dose or repeated intermittent administration of alcohol. Three graded levels of vitamin C in their diet were applied: traces (none added), 0.05 % (w/w) and 0.5 % (w/w). The basal diet was nutritionally adequate, with 14.4 % of protein, 39.3 % of fats and 46.3 % of carbohydrates (in percentage of the dietary energy value). The experimental period lasted five weeks.

In the final part of the first experiment (the model of acute ethanol intoxication), the animals (13 in each group) were given one i.p. dose of ethanol (dissolved in saline, 30 % w/w) in a dose of 3 g per kg of body weight 24 hours before killing. In the second experiment (the model of long-term chronic intoxication), the guinea-pigs (17 in each group) received ethanol i.p. twice a week in a dose of 1 g per kg of body weight. Twenty-four hours before decapitation, the animals received the same amount of ethanol as those in the first experiment.

Tissue concentrations of vitamin C were determined according to Roe and Kuether (1943). The residual contents of ethanol and acetaldehyde (the first metabolic product of ethanol) were estimated in the liver and brain using gas-chromatography as described by Brien and Hoover (1980). The enzymatic activities of liver aminoacyltransferases (aspartate- and alanine transferase, AST, ALT) in the serum were evaluated by means of kit Bio-La-Test PND 50-37 082. The content of total cholesterol and triacylglycerols in the serum, as well as of triacylglycerols in the liver, were determined enzymatically by means of Bio-La-Test kits PND 50-398-82 and PND 50-442-82, respectively. Cholesterol in the liver was estimated colorimetrically according to Libermann-Burchardt (Krondl 1962).

The statistical significance of differences between the results obtained in experimental (i.e. supplemented) and control (unsupplemented) groups was assessed by Student's t-test.

Results and Discussion

There were no significant differences in body weight between experimental and control groups. In both experiments the five-week period of differentiated vitamin C intake resulted in the highly significant differences between tissue concentrations of ascorbate in the liver (Table 1).

Table 1. Ascorbic acid, ethanol and acetaldehyde contents in tissues of guinea-pigs saturated with graded doses of vitamin C and intoxicated with ethanol

Group		A	B	C
Experiment I (Short-term intoxication)				
Liver	- ascorbic acid (mg/100g)	2.55±0.44**	7.75±0.61**	16.28±0.85
	- ethanol (mg/100g)	2.20±0.14**	1.95±0.08**	1.25±0.07
	- acetaldehyde(mg/g)	1.19±0.06**	0.74±0.05**	0.44±0.03
Brain	- ethanol (mg/100g)	1.90±0.1**	1.76±0.10	1.78±0.10
	- acetaldehyde (mg/g)	1.86±0.07**	1.62±0.04**	1.38±0.04
n		13	13	13
Experiment II (Long-term intoxication)				
Liver	- ascorbic acid (mg/100g)	3.80±0.37**	11.67±0.63**	20.93±0.85
	- ethanol (mg/100g)	1.46±0.08**	1.23±0.09**	0.81±0.06
	- acetaldehyde(mg/g)	0.47±0.02**	0.45±0.04**	0.33±0.02
Brain	- ethanol	1.26±0.12**	0.80±0.10**	0.64±0.11
	- acetaldehyde	0.42±0.01	0.30±0.26**	0.17±0.10
n		10	10	10

*Group A - no added vitamin C, B - 0.05 % vitamin C in the diet, C - 0.5 % vitamin C in the diet. Results are presented as the mean ± S.E.M. ** significantly different from the group C ($P<0.001$), n - number of animals in the group.*

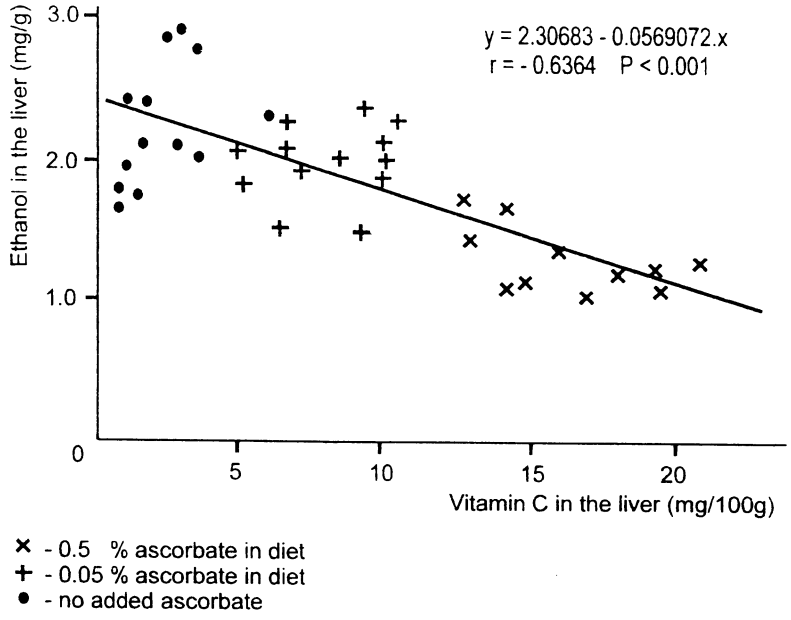

Fig. 1. *Regression curve reflecting vitamin C and residual ethanol in the liver of alcoholized guinea-pigs.*

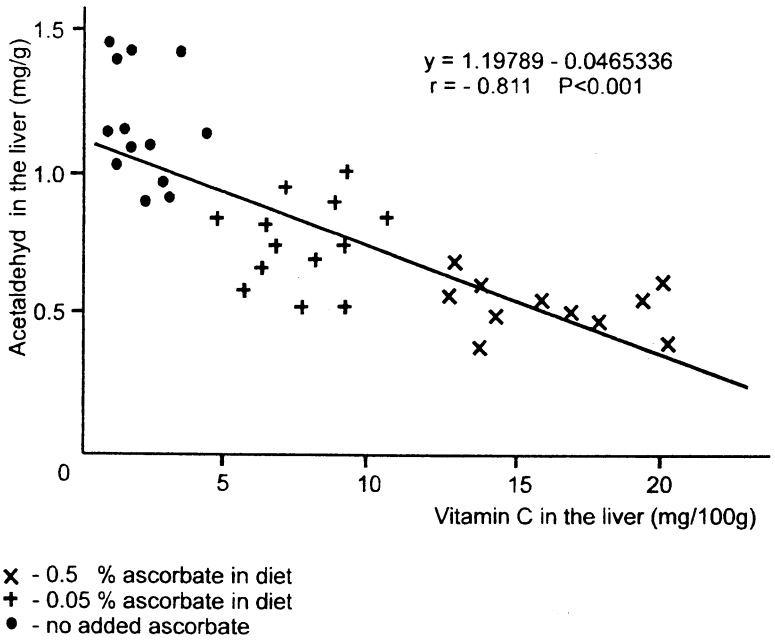

Fig. 2. *Regression curve reflecting vitamin C and residual acetaldehyde in the liver of alcoholized guinea-pigs.*

Determinations of the residual ethanol content in the tissues of animals treated in the first experiment (Table 1) revealed a significant decrease of this noxa in the liver of highly vitamin C-saturated animals in comparison with the control group. The acetaldehyde concentration in the liver as well as in the brain correlated with the vitamin C-status similarly as the ethanol residuum. In the second experiment, the elimination rate of ethanol and acetaldehyde from the liver (Fig. 1) positively correlated with the liver ascorbate concentration.

Table 2. Aspartate and alanine aminoacyl transferase (AST, ALT) activities in the serum and triacylglycerol (TG) and cholesterol (CH) contents in the serum and liver of guinea-pigs saturated with graded doses of vitamin C and intoxicated with ethanol

Group		A	B	C
Experiment I (Short-term intoxication)				
Serum	- AST (μkat/l)	0.58±0.02*	0.51±0.05	0.48±0.06
	- ALT (μkat/l)	0.70±0.06*	0.64±0.05	0.51±0.04
	- TG (mmol/l)	1.09±0.07*	0.84±0.24	0.82±0.06
	- CH (mmol/l)	3.66±0.55	3.56±0.24	3.34±0.19
Liver	- TG (mmol/kg)	117.6±7.2*	109.0±7.5	103.1±7.7
	- CH mol/kg)	13.12±0.76*	10.98±0.55	10.02±0.61
n		13	13	13
Experiment I (Long-term intoxication)				
Serum	- AST (μkat/l)	0.48±0.41*	0.40±0.04	0.36±0.03
	- ALT (μkat/l)	0.45±0.03*	0.39±0.02	0.39±0.03
	- TG (mmol/l)	0.88±0.07*	0.87±0.05*	0.70±0.05
	- CH (mmol/l)	3.17±0.33*	2.59±0.25	2.01±0.23
Liver	- TG (mmol/kg)	90.4±7.2*	74.5±9.3	70.4±8.7
	- CH (mmol/kg)	12.0±0.8*	11.0±0.89*	8.16±0.35
n		17	17	17

*Group A – no added vitamin C, B – 0.05 % vitamin C in the diet, C – 0.5 % vitamin C in the diet. Results are presented as the mean ± S.E.M. * statistically significant difference from the group C ($P<0.05$), n – number of animals in the group.*

AST and ALT in the serum of animals with poor vitamin C-status (Table 2) reached the highest values in comparison with the vitamin C-supplemented animals. The AST and ALT activities indicate decreased injurious activity of ethanol and acetaldehyde towards the liver parenchyma in both C vitamin-supplemented groups compared to the controls. The increased elimination of ethanol and acetaldehyde in tissues with high vitamin C concentrations seems to be the basic mechanism of ethanol-ascorbate interactions and of vitamin C-mediated defense against ethanol and acetaldehyde toxicity.

Ethanol abuse is known to alter lipid metabolism, esp. to elevate total and HDL-cholesterol and triacylglycerol levels in the serum and to develop fatty liver (Lieber 1994a). In our experiments, increased triacylglycerols were found in the serum and total cholesterol was elevated in the liver of vitamin C-unsupplemented alcoholized group in comparison with both vitamin C-supplemented groups (Table 2). The loading of animals with ethanol led to a deterioration of lipid metabolism, however, the high vitamin C saturation of body tissues significantly limited these alterations in terms of the protective, hypolipidaemic activity of ascorbate.

The investigations that have tried so far to solve the possible impact of vitamin C on ethanol metabolism have brought about very ambiguous results in both humans and animals. In male human volunteers, the clearance of ethanol from the blood positively correlated with the leukocyte vitamin C content (Bonjour 1979). In another experiment, however, neither the daily administration of 20 mg vitamin C for 10 days nor the administration of 600 mg ascorbate daily for five days changed the rate of alcohol metabolism in healthy men (Bonjour 1979). In rats pretreated with ascorbate (440 mg/kg body weight) and administrated i.p. with 0.75 g ethanol per kg body weight blood ethanol concentrations were significantly reduced compared to the controls (without ascorbate) (Bonjour 1979). No difference was found in the rate of ethanol elimination from the blood of scorbutic and non-scorbutic guinea-pigs after an injection of 2.5 g ethanol/kg body weight (Dow and Goldberg 1975). Other investigators have failed to demonstrate any specific effect of ascorbate on the activity of alcohol and/or aldehyde dehydrogenase (Jörnwal 1994).

Our results imply that large doses of vitamin C, highly exceeding antiscorbutic ones, have a pronounced stimulating effect on the catabolism of both alcohol and acetaldehyde in guinea-pigs. The more marked decrease of these noxious substances in the liver and in the brain as a result of C-vitamin supplementation is of special significance because both organs are very sensitive to their toxic action.

The metabolic breakdown of ethanol proceeds *via* two main pathways, the most important being the NAD^+-dependent enzymatic alcohol- and aldehyde dehydrogenation to acetic acid (Jörnwall 1994). The alternative course of ethanol metabolism in man as well as in rodents involves a cytochrome P450-dependent, microsomal ethanol-oxidizing system (CYP 2E1) (Roberts *et al.* 1995). This CYP 2E1 enzyme is induced in case of long-term chronic and/or short-term ethanol intoxication. Thus, it may contribute to the increased rate of ethanol breakdown in cases of high ethanol consumption (Lieber 1994b). In general, ascorbate is believed to act as an activating factor for CYP 2E1 (Peterson *et al.* 1982) and, similarly, it may account for the accelerated oxidative catabolism of ethanol in the liver. Ethanol oxidation through the activated microsomal ethanol-oxidizing system then leads to the production of toxic radical intermediates such as the hydroxyethylradical (derived from the ethanol molecule) (Moore *et al.* 1995) and the superoxide anion which takes part in the deleterious effects involved in ethanol toxicity (Lieber 1997). Ascorbate primarily scavenges these reactive species (Nordmann 1994) and the irreversible oxidative destruction of ascorbate resulting from this reaction implies an increased metabolic rate of vitamin C. For this reason, an enhanced need for vitamin C may be a consequence of ethanol metabolic detoxification.

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