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Influence of Vitamin C Status on the Metabolic Rate of a Single Dose of Ethanol-1-^{14}C in Guinea Pigs

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Summary

The rate of oxidative metabolism after a single i.p. dose of ethanol-1-^{14}C was studied in male guinea pigs, previously treated with two different levels of vitamin C (traces or 0.5 g/100 g) in their diet for 5 weeks. While the body weight did not differ between these two groups after 5 weeks of the dietary regimen, the vitamin C concentration in the liver was five times higher in the group with the high vitamin C intake. The cumulative amounts of breathing $^{14}CO_2$ measured at short time intervals during 24 hours after an ethanol-^{14}C injection (23 mg ethanol and 160 kBq per kg body weight or 2.35 g ethanol and 165 kBq per kg body weight in a parallel experiment) were significantly different. The half-time of ethanol turnover reached a value of 5.1 h versus 6.9 h (9.9 vs 14.4 h in a parallel experiment) in the high and low saturated group respectively. The long-term pretreatment of guinea pigs with large doses of vitamin C accelerated ethanol metabolism. Improvement of the redox state and activation of the cytochrome P450 system in vitamin C-supplemented organism are considered to be the reason for the increased ethanol catabolism.

Key words

Vitamin C • Ethanol turnover • Ethanol oxidative metabolism

Introduction

It has long been known that alcohol abuse is linked to many adverse effects including those damaging the drinker's nutritional status (Lecomte *et al.* 1994). On the other hand, many dietary factors are supposed to protect the organism against the health-injuring effect of alcohol and its metabolites, especially that of acetaldehyde (Sprince *et al.* 1978). Vitamin C (ascorbic acid) has usually been mentioned as a strongly effective nutrient in counteracting alcohol and acetaldehyde toxicity (Susick *et al.* 1986, Susick and Zannoni 1987). However, the biochemical principle of this ascorbic acid action has not yet been elucidated nor definitely confirmed (Seitz and Suter 1994).

The generally accepted opinions concerning the ascorbate-alcohol relationships have been based on the findings that increased alcohol dehydrogenase activity was reported in guinea pigs fed a high-vitamin C diet (Bonjour 1979). A more rapid elimination of ethanol from the blood after ethanol ingestion was repeatedly reported to be related to the vitamin C body pool in both healthy human volunteers (Majumdar *et al.* 1983) and in experimental animals (Ginter *et al.* 1998). L-ascorbate has been suggested to activate an alternate ethanol metabolic pathway – the microsomal ethanol oxidizing

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system (Zerilli *et al.* 1995), which appears to be the result of excessive or chronic ethanol intoxication (Song and Cederbaum 1996). As NADPH is a co-enzyme activating the cyclic ethanol dehydrogenation process, ascorbic acid appears to influence favorably the reductive renewal of NADPH.

On the other hand, other investigations have failed to demonstrate any specific effects of ascorbate on the activity of ethanol metabolizing enzymes as well as on the toxic properties both of ethanol and metabolic intermediates such as acetaldehyde and acetic acid (Dow and Goldberg 1975, Chen *et al.* 1990). The aim of the present report was to elucidate the relationship between C-vitamin status and the rate of ethanol turnover in experimental animals. The original methodological approach to this problem was to administer ^{14}C-labelled ethanol to guinea pigs and to follow its elimination from the body.

Method

Twelve male guinea pigs of laboratory breed were maintained for 5 weeks on a nutritionally adequate diet (with exception of vitamin C). The vitamin C content in the diet was below the physiological requirements for guinea pigs (i.e. under 0.01 g/100 g). Six animals were fed this low C-vitamin diet (group H, hyposaturated), whereas the remaining six animals received a diet supplemented with ascorbic acid to the concentration 0.5 g/100 g diet – group S (saturated). The initial body weight of the experimental animals was about 330 g.

At the beginning of the sixth week of the experiment, all guinea pigs were administered a single i.p. dose of 1 % 1-^{14}C-ethanol solution in saline (i.e. approximately 23 mg ethanol/kg body weight). The radioactivity applied was 148-178 kBq per kg body weight. Immediately after the injection, the animals were placed individually into glass metabolic cages with forced air circulation. After 2, 4, 6, 8, 12 and 24 hours the breathing air was separated and absorbed in an ethanolamine solution in order to evaluate the radioactivity of exhaled $^{14}CO_2$. A method of scintillation spectrometry was used for determination of ^{14}C in the exhaled carbon dioxide (Nemec *et al.* 1971).

In the second experiment, the same animals with the differentiated vitamin C intake were used after they had eliminated all the radiocarbon administered in the previous assay. A substantially larger dose of ethanol was administered in this experimental series: 30 % 1-^{14}C-ethanol in saline containing approximately 2.35 g alcohol per kg body weight, the radioactivity value being 158-173 kBq per kg body weight. The quantitative analysis of $^{14}CO_2$ in breathing air was performed in the same way as in previous experiment. The amounts of $^{14}CO_2$ estimated in separate time intervals were used for evaluating the pharmacokinetic parameters of ethanol metabolism (Crabb *et al.* 1987) in relation to the different vitamin C status of the experimental animals.

After decapitation, the concentration of ascorbic acid in the liver was determined spectrophotometrically according to Roe and Kuether (1943). The statistical significance of the differences between the groups H and S was assessed by Student's t-test.

Table 1. Body weight of the experimental animals, vitamin C content in the liver, dose of ethanol and radioactivity of ^{14}C-1-ethanol administered

Concentration of ^{14}C-ethanol solution (% v/v)	Experimental group	N	Body weight (g)	Vitamin C content in the liver (mg/100 g)	Dose of ethanol (mg/100 g b.w.)	Dose of ^{14}C (kBq/100 g b.w.)
1	H	6	428 ± 11		2.39 ± 0.04	17.5 ± 0.3
	S	5	433 ± 7		2.30 ± 0.07	14.8 ± 0.4
30	H	6	413 ± 18	3.8 ± 0.5	234.5 ± 0.6	17.3 ± 0.1
	S	6	420 ± 11	21.8 ± 0.4	235.2 ± 2.4	15.8 ± 0.2
				$p < 0.001$		

Group H - hyposaturated with ascorbic acid, Group S - saturated with ascorbic acid. Results are means ± S.E.M. N is number of animals in the group

Results

The body weight, ascorbic acid content in the liver, the injected amount of alcohol and the ^{14}C-dose applied in the form of 1-^{14}C-ethanol are summarized in Table 1.

The body growth of experimental guinea pigs in the hyposaturated (H) and saturated group (S) proceeded at a similar rate throughout the 6-week experimental period despite the different intake of vitamin C. The ascorbic acid content in the liver was, however, greatly different (3.8 mg and 21.8 mg/100 g, respectively, $p<0.001$) in the two experimental groups.

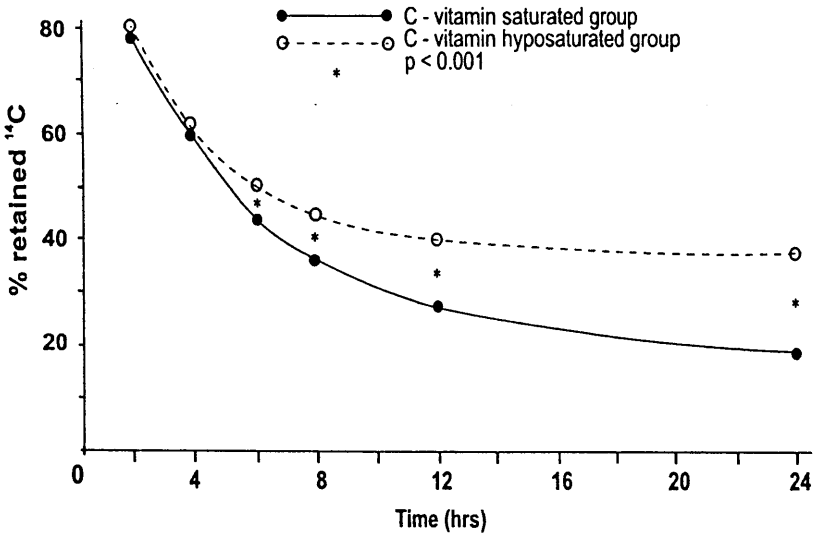

Fig. 1. *The dynamics of ^{14}C depletion (expressed as mean % ^{14}C retained) in the guinea pigs after dosing a small amount of 1-^{14}C-ethanol*

The expired amounts of $^{14}CO_2$ correspond to the total oxidative destruction of the ethanol molecule during its metabolic turnover in the body. The time-course of ^{14}C depletion in the first experiment (a small dose of ethanol) is presented as the residual ^{14}C in the body at consecutive short-time intervals following ^{14}C-ethanol administration (Fig. 1). At the last time interval of the experimental period (24 h) the amounts of ^{14}C-ethanol (and/or its metabolites) retained in the body of H and S group differed significantly. This indicates that ethanol catabolism and excretion is markedly slower in the C-vitamin hyposaturated group in comparison with the group on the high ascorbate intake.

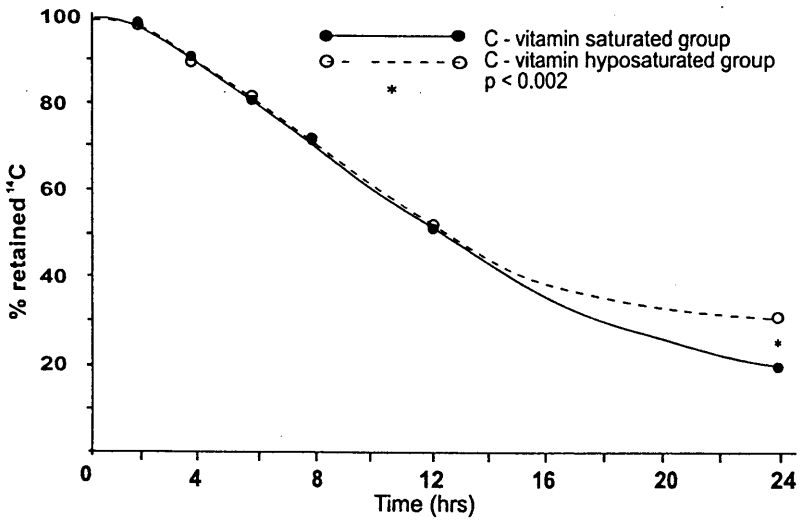

Fig. 2. *The dynamics of ^{14}C depletion (expressed as mean % ^{14}C retained) in the guinea pigs exposed to a large dose of 1-^{14}C-ethanol*

The dynamics of ^{14}C excretion in animals subjected to large amount of ^{14}C-ethanol (Fig. 2) is similar to those in the previous experiment. The more rapid turnover of ethanol after higher vitamin C

saturation of the animals has been confirmed by pharmacokinetic calculations of ethanol half-time elimination: 9.87 h vs 14.42 h, this difference being statistically significant ($p<0.01$).

Discussion

The carbon dioxide expired is considered to be the end-product of ethanol oxidation. While the first step in ethanol turnover includes its oxidation to acetaldehyde by a cytosol and/or mitochondrial enzyme alcohol dehydrogenase (ADH, cooperating with NAD^+ cofactor, Eaton *et al.* 1997), in the mitochondria, a highly effective enzyme aldehyde dehydrogenase (ALDH, with the same cofactor NAD^+) oxidizes acetaldehyde to acetic acid which may be ubiquitous in circulating blood (Lieber 1997). A large proportion of the acetate resulting from ethanol metabolism is oxidized to CO_2 (after ATP-dependent metabolic activation to acetyl-coenzyme A), mainly by decarboxylation in the tricarboxylic acid cycle (Lieber 1994).

The metabolic pathway starting from ethanol and terminating as acetate may proceed by an alternate oxidative action of catalase in the presence of H_2O_2 (Chen *et al.* 1992). The so-called microsomal ethanol oxidizing system, MEOS (Zerilli *et al.* 1995), and the microsomal aldehyde oxidizing system, MAOS (Zimatkin and Deitrich 1997, Kunitoh *et al.* 1997), represent alternative substrate-inducible, cytochrome P450 containing complexes which also participate in ethanol oxidation. Their significance increases after excessive and/or long-term ethanol intoxication.

The differences in the amount of expired $^{14}CO_2$ found in both experiments are very marked. They serve as evidence for the stimulating role of ascorbate in ethanol metabolism implying a detoxifying function of this vitamin in relation to alcohol toxicity.

However, experimental data concerning the role of ascorbate in the metabolism of ethanol and other drugs as well as contemporary opinions on this issue are rather controversial. With the exception of the substrate and products pool, the kinetics of ethanol flux through ADH and ALDH is dependent on the relation NAD^+/NADH (Azzalis *et al.* 1994). According to the opinion of some investigators, ascorbate stimulates the ADH biosynthesis and activity (Bonjour 1979). However, these assumptions have not been unanimously accepted because no causative relation between ascorbate concentration in the liver and ethanol metabolic turnover had been confirmed in animals which do not synthesize vitamin C (Chen *et al.* 1990).

Besides apo-enzyme disposal, the NAD^+/NADH ratio plays a crucial role in the rate of ethanol oxidation (Bello *et al.* 1994). The increased NADH concentration in hepatocytes resulting from dehydrogenases activities has to be compensated by the re-oxidation of NADH through the respiration chain in mitochondria which induces a larger need for ADP in order for the oxidative phosphorylation to proceed. A sufficiently high pool of NAD^+ is necessary for the normal course of the tricarboxylic acid cycle which is the main pathway for acetyl-coenzyme A utilization. The favorable effect of ascorbate on ethanol oxidation, reflected by the results of our experiments, seems to be related to the improvement of a process converting NADH to NAD^+. This effect may be linked to the redox activity of ascorbate in an intracellular compartment of the experimental guinea pigs treated with high doses of vitamin C.

A potential link between NADH and $NADP^+$ is also assumed. This stimulates electron and H^+ transport leading to the NADPH formation (Cornell *et al.* 1979). NADPH-cytochrome oxidoreductase is a crucial component of MEOS and MAOS (Kunitoh *et al.* 1997), i.e. of metabolic pathways playing an important role in the case of excessive or chronic alcohol intoxication.

The activities of MEOS and MAOS are supposed to be controlled by ascorbate levels in hepatocytes (Crabb *et al.* 1987). This biochemical principle can be considered as an alternative mechanism activating ethanol oxidative destruction in guinea pigs treated with a large dose of ethanol (the second experiment).

In conclusion, a large dietary uptake of vitamin C leading to high ascorbate concentrations in the liver is associated - apparently in a causative manner – with accelerated oxidative metabolism of alcohol. This implies that this nutrient has an ameliorative effect on ethanol toxicity. Therefore, an increased need for vitamin C may be a metabolically conditioned consequence of ethanol abuse in man.

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