Effect of Treatment with DL-carnitine after Acute Alcoholization in Rats

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Summary

Acute ethanol consumption leads to the formation of free radicals. Among other functions, carnitine has an important antioxidant role and chronic ethanol use leads to carnitine deficiency. The objective of the present study was to determine the variation in the carnitine pool (free cernitine plus its acylated derivates) and the hepatic oxidative stress occurring in the presence of acute ethanol administration followed by treatment with carnitine in rats. Male Wistar rats weighing approximately 60 g were divided at random into four groups of 7 animals each, i.e., group receiving carnitine, group receiving carnitine plus ethanol, group receiving ethanol alone, and untreated control. Acute administration of ethanol and/or carnitine did not change the total amount of carnitine and its derivates in plasma but did alter their profile with the free carnitine increasing to over 75%, while the mean percentage of free carnitine in the control group was 33.2%. There was marked carnitine excretion in the groups treated with DL-carnitine. Higher lipid peroxidation was detected in the groups receiving carnitine, with the maintenance of vitamin E. We conclude that the administration of DL-carnitine after an episode of alcohol intoxication has no beneficial effect in terms of hepatic oxidative stress.

Introduction

Carnitine is a quaternary amine synthesized in the organism (liver, kidneys, brain) from two essential amino acids, lysine and methionine, with synthesis requiring the presence of iron, ascorbic acid, niacin and vitamin B_6 (*Coelho et al., 2005*). Carnitine is essential for the normal oxidation of fatty acids by the mitochondria. Among the known effects of the administration of carnitine or its derivatives is antioxidant protection preventing the formation of reactive oxygen species and free radical scavenging in the aggression provoked by ethanol administration (*Dokmeci et al., 2005*). Carnitine has been used as a supplement in various clinical situations such as chronic obstructive pulmonary disease in order to improve the aerobic capacity of these patients (*Silva et al., 2003*).

When carnitine is transported together with methanol there is stimulation of cytochrome P450 (CYP2E1) by about 170% over a period of 24 hours and by 145% over a period of 96 hours (*Olszowy et al., 2006*).

A marked reduction of carnitine concentration occurs during the chronic use of ethanol (*Sakvarelidze, 2006*), probably related to antioxidant metabolism since acute ethanol administration is known to lead to an increase in free radicals (*Jordao et al., 2004*).

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While studies have focused on treatment with carnitine before the use of ethanol, there are no data about the efficacy of its use after a period of acute alcohol intoxication (*Dokmeci et al., 2005; Calabrese & Rizza, 1999; Sachan et al., 1984, 2002*).

On this basis, the objective of the present study was to assess the variation in the carnitine pool and the effect of treatment with carnitine on hepatic oxidative stress after acute ethanol administration in rats.

Materials and Methods

Male Wistar rats weighing about 60 g were obtained from the Central Animal Facilities of the Ribeirão Preto Campus, USP and left in the animal facilities of the Department of Internal Medicine, FMRP/USP for a 3-day period of adaptation, with free access to water and commercial rat chow (Nuvilab® Cr-1, Nuvital Nutrientes Ltda., Colombo, Brazil). The animals were then divided at random into four groups of 7 animals each:

Control Group: no treatment

Carnitine Group: supplemented with 50 mg/kg DL-carnitine (intragastric solution administered by gavage)

Ethanol Group: acute 5 g/kg dose (50% intragastric solution administered by gavage)

Ethanol + Carnitine Group: acute ethanol 5 g/kg dose (50% intragastric solution administered by gavage) + supplementation with 50 mg/kg DL-carnitine (intragastric solution administered by gavage).

The animals were left in individual metabolic cages for 24 hours for urine collection and then sacrificed by decapitation. Blood was collected for biochemical determination of free and total carnitine by an adaptation of the method proposed by Xia & Folkers (*1991*) as follows: 250 µL of plasma in a test tube were deproteinized by the addition of 10 times the volume of pure ethanol. After shaking for 3 minutes in a vortex mixer, the tubes were centrifuged at 3500 rpm for 10 minutes. The supernatant was transferred to another tube, two additional washes with 500 µL ethanol were performed and the supernatants were added to the first. The supernatants were then evaporated to dryness under a nitrogen gas flow at room temperature and 1 mL water was added for carnitine resuspension and free and total carnitine was measured by the same method as above (*Xia & Folkers, 1991*).

For the determination of oxidative stress parameters, the liver of each animal was removed, weighed and immediately immersed in liquid nitrogen and stored at -40ºC for later analysis of reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS) expressed as malondialdehyde (MDA), and vitamin E using standard methods employed in our laboratory (*Jordao et al., 2004*).

Results

The concentrations of free, total and acylcarnitines in rat plasma are listed in Table 1. It can be seen that acute administration of ethanol and/or carnitine did not change the total carnitine levels in plasma, but did alter the profile of free carnitine, which was increased to more than 75% in relation to total carnitine. Plasma carnitine concentration was significantly lower in the control group ($P<0.05$), possibly reflecting a state of organic equilibrium with no need for changes in energy metabolism, with a mean 33.2% value in relation to total carnitine.

Table 2 shows the urinary carnitine excretion in the groups studied. It can be seen that there was a marked excretion in the groups treated with DL-carnitine, with no detection in the Control and Ethanol groups. Also, carnitine excretion was significantly greater in the alcoholic group ($P<0.05$). The same excretion pattern was observed when the values were corrected for 24-h urinary volume (Table 3).

Data concerning hepatic oxidative stress are summarized in Table 4. The highest lipid peroxidation values were detected in the groups receiving carnitine, while the opposite was observed for GSH. Vitamin E levels were unchanged in all groups.

Table 1. Plasma concentrations of free and total carnitine and of acylcarnitine in rats.

Groups / [ ] µmol/L	Control	Carnitine	Ethanol	Ethanol + Carnitine
Free	6.9±3.8	17.2±4.0*	12.3±4.3	19.3±5.6*,**
Total	20.8±7.1	23.3±8.8	16.3±3.0	25.6±7.2
Acylcarnitines (by difference)	14.3±9.5	7.7±5.4	5.2±3.2	6.3±4.5
% free in relation to the total	33.2	73.8	75.5	75.4

Control Group: no treatment
Carnitine Group: supplemented with 50 mg/kg carnitine (intragastric solution)
Ethanol Group: acute dose of 5 g/kg (50% intragastric solution)
Carnitine + Ethanol Group: supplemented with 50 mg/kg carnitine (intragastric solution)+ an acute dose of 5 g/kg (50% intragastric solution)
* Significant difference from Control Group, $P<0.05$
** Significant difference from Ethanol Group, $P<0.05$

Discussion

The data obtained in the present study show that the animals treated with DL-carnitine had a significant increase in plasma free carnitine, but with no increase in acylcarnitines.

In studies using methanol, a considerable increase in free carnitine and acylcarnitine levels was observed as early as 30 minutes after intraperitoneal carnitine administration, with an increase as high as 200% in relation to basal levels (*Olszowy et al., 2006*).

Another pioneering study regarding this topic was that by Kondrup and Grunnet (*1973*), who reported changes in the carnitine profile in animals receiving ethanol in a chronic or acute form. The animals subjected to acute administration and sacrificed 3 hours after receiving 3 g ethanol/kg body weight showed an increase in carnitine levels (*Kondrup & Grunnet, 1973*).

The urinary excretion detected here in the groups supplemented with carnitine may be a possible explanation for the lack of an increase in total carnitine levels. Other investigators believe that 70–90% of orally administered carnitine may be lost due to bacterial degradation in the alimentary tract and recommend the use of intraperitoneal administration in order to reach higher levels (*Olszowy et al., 2006*). However, it should be emphasized that, even though total plasma carnitine levels did not differ between the carnitine-supplemented and control groups, their distribution was modified, with more than 75% of carnitine being in the free form in the carnitine-supplemented group. It is difficult to explain this

Table 2. Urinary concentrations (μmol/L) of free and total carnitine and of acylcarnitines in rats.

Type \ Groups	Control	Carnitine	Ethanol	Ethanol + Carnitine
Free	nd	2.9±1.4	nd	95.4±48.8*
Total	nd	41.9±34.5	nd	122.0±52.4*
Acylcarnitines (by difference)	nc	39.1±33.8	nc	26.6±20.1

nd = not detected
nc = not calculated
* $p<0.05$; compared to Carnitine Group

Table 3. Carnitine (μg) excreted in 24 h into urine.

Type \ Groups	Control	Carnitine	Ethanol	Ethanol + Carnitine
Free	nd	3.8±2.2	nd	164.7±62.0*
Total	nd	54.7±38.4	nd	212.0±55.4*
Acylcarnitines (by difference)	nc	50.9±37.0	nc	47.3±42.9

nd = not detected
nc = not calculated
* $p<0.05$; compared to Carnitine Group

result, but we may suggest that a negative feedback in the presence of supplementation reduced the endogenous synthesis of carnitine, thus increasing its mobilization from peripheral tissues to plasma in the free form. The expected percentage of acylcarnitine for free carnitine is 40% in human plasma (*Meyburg et al., 2001*) and 35% in Wistar rats (*Clouet et al., 1996*).

The groups treated with carnitine showed the worst rates of hepatic oxidative stress (Table 4) related to increased lipid peroxidation and GSH consumption. We postulate that carnitine administration blocks alcohol dehydrogenase by competition with NAD, causing greater activity of the microsomal system of ethanol oxidation, which increases the production of free radicals leading to oxidative stress (*Calabrese*

Table 4. Parameters of hepatic oxidative stress.

Parameter \ Groups	Control	Carnitine	Ethanol	Ethanol + Carnitine
TBARS (nmol/mg protein)	0.16±0.02	0.34±0.08*,**	0.19±0.02	0.30±0.06*,**
GSH (μmol/g protein)	61.50±9.00	6.04±1.40*	46.99±5.48*	7.28±1.51*,**
Vitamin E (μmol/g tissue)	32.34±30.20	23.45±11.54	17.40±4.63	20.73±2.25

* Significant difference from Control Group, P<0.05
** Significant difference from Ethanol Group, P<0.05

& Rizza, 1999; Sachan et al., 1984; Czech et al., 2004).

The present data permit us to conclude that the administration of DL-carnitine after an episode of alcohol intoxication, represented here by an acute dose of ethanol (5 g/kg body weight), has no beneficial effect against hepatic oxidative stress. In conclusion, we believe that acute ethanol administration leads to significant changes in the profile of plasma carnitine, especially in relation to total and free carnitine, and that these changes should be better studied in terms of dose and time and mode of administration.

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