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Protective effect of treatment with thiamine or benfotiamine on liver oxidative damage in rat model of acute ethanol intoxication

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ABSTRACT

Aims: The aim of this study was to evaluate possible beneficial effects of treatment with thiamine or benfotiamine in an animal model of acute ethanol intoxication.
Main methods: Thirty male Wistar rats were separated at random into three groups of 10 animals each: Ethanol (E), Ethanol treated with thiamine (T) and Ethanol treated with benfotiamine (BE). Rats were gavaged with single dose of ethanol (5 g/kg, 40% v:v). After 30 min of ethanol gavage the animals were treated with thiamine or benfotiamine. Six hours after first gavage, the animals were euthanized and blood and liver samples were collected for ethanol and oxidative stress biomarkers quantification.
Key findings: Serum ethanol levels were higher in animals treated with thiamine or benfotiamine while hepatic alcohol levels were higher in animals of the group treated with benfotiamine comparing to controls or thiamine treated groups. The lipid peroxidation biomarkers were diminished for the groups treated with thiamine or benfotiamine comparing to E animals. Concerning protein oxidative damage parameters, they were enhanced for animals treated with benfotiamine in relation to other groups.
Significance: In conclusion, the treatment with thiamine or benfotiamine even 30 min after the massive dose of ethanol has proven to be beneficial against liver damage. Improved results were obtained with benfotiamine in relation to oxidative damage from aqueous compartments.

1. Introduction

Ethanol metabolism produces free radicals and reactive oxygen species with consequent consumption of antioxidants leading to an imbalance between oxidants/antioxidants, known as oxidative stress. Several research groups have focused on the study of liver diseases caused by alcohol, since this is the organ responsible for about 95% of its catabolism [1]. The hepatic metabolism of ethanol is carried via three enzymatic pathways: pathway of alcohol dehydrogenase, the microsomal ethanol oxidation system (CYP2E1), and catalase [2,3]. While these metabolic pathways are well elucidated in the literature, little attention is given to the quantification of ethanol in liver although this can be an important finding to know the metabolizing status of alcohol in this organ.

During the transformation of ethanol to acetaldehyde via CYP2E1 there is an overproduction of free radicals and reactive oxygen species, mainly in the forms of superoxide anion (O_2^-), hydrogen peroxide (H2O2) and hydroxyethyl radical ($CH_3CH^{(\bullet)}$—OH) [4–6]. These molecules are highly reactive and, as acetaldehyde, attack macromolecules, causing impairment or loss of function and, ultimately, cell death [7].

The use of antioxidants may help balance the hepatic antioxidant system reducing the deleterious effects caused by oxidative stress [8].

Thiamine, the vitamin B1, is a water soluble vitamin that plays essential role on energetic metabolism from carbohydrates. Benfotiamine is a weak soluble in water pro-vitamin B1 substance that displays better absorption and bioavailability compared with common pharmaceutical form available, thiamine hydrochloride, when administered orally, even after massive ethanol administration [12]. Thiamine therapeutic replacement is postulated in patients with Wernicke encephalopathy, especially alcoholics, due to deficiency of this vitamin in this particular group, because of poor dietary habits and a reduced absorption due to changes in the gastrointestinal system [9,10]. Hypothetically, thiamine supply could increase the metabolism of ethanol by restoring the microsomal ethanol oxidizing system (MEOS).

Studies from our group have previously demonstrated some beneficial effects on intravenous administration of thiamine in an animal model of acute ethanol intoxication and more recently the high

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bioavailability of benfotiamine in rats acutely alcoholized [11,12]. In humans, benfotiamine has been tested as oral treatment of alcoholic polyneuropathy with great improvement of the symptoms [13].

The National Institute on Alcohol Abuse and Alcoholism (NIAAA) defines binge drinking as a pattern of drinking that brings blood alcohol concentration (BAC) levels to 0.8 g/L, typically occurring after 4 drinks for women and 5 drinks for men in about 2 h [14,15]. In experimental models this can be achieved by forced administration of a massive dose of ethanol by gavage since animals do not voluntarily consume alcohol at concentrations that produce intoxication [15]. It has been showed that, as the chronic models, the acute ethanol intoxication produces an increase in hepatic oxidative stress.

In the present study we evaluate the effects of the administration of benfotiamine or thiamine on the improvement of hepatic oxidative damage and ethanol influx in a model of acute ethanol intoxication

2. Material and methods

2.1. Animals and experimental protocol

Thirty male Wistar rats, weighing 270–333 g, were obtained from the Central Animal Facilities of the Ribeirão Preto Campus, University of São Paulo, and allowed to acclimate for 1 week in the animal facilities of the Department of Internal Medicine, Faculty of Medicine of Ribeirão Preto, University of São Paulo, under controlled conditions of a 12-h light: dark cycle and temperature of 24 ± 2 °C in individual cages with free access to food and water. The experimental protocol was approved by the Animal Research Ethics Commission (protocol no. 152).

The animals were separated at random into three groups of 10 animals each: Ethanol (E + S), Ethanol treated with thiamine (E + T) and Ethanol treated with benfotiamine (E + BE). In the dark period of the previous day (10:00 p.m.) the chow was withdrawal to ensure that the stomach was not filled preventing reflux and aspiration of fluid into lungs and, mimic the eating habits frequently encountered in alcoholics. Next day, at 6:00 a.m. the rats were gavaged with single dose of 40% (w:v) ethanol in aqueous solution (5 g/kg of body weight) and, after 30 min each group received a second gavage to delivery 100 mg/kg of thiamine (E + T), in an aqueous solution, or benfotiamine (E + BE), in an aqueous dispersion, except for E + S group that received a saline sham gavage. Animals were left in cages with food and water ad libitum but due to sedation caused by ethanol none of them sought for food.

Six hours after the first gavage, the animals were euthanized and blood and liver samples were collected, weighed, frozen immediately in liquid nitrogen and stored at −40 °C until analysis.

2.2. Serum and hepatic ethanol quantification

Ethanol was quantified in serum and liver homogenates by previous validated gas chromatography method [16]. The hepatic ethanol concentration was multiplied by the respective liver mass to achieve total content of ethanol in this organ. Then, the percentual of ethanol relative to the initial doses was calculated.

2.3. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

To check for liver damage were measured enzymes AST and ALT by colorimetric reaction and spectrophotometric reading (UV–Vis Mod Q98U Quimis®, Diadema, SP, Brazil), using commercial kit (Labtest Diagnostica, Lagoa Santa, MG, Brazil).

2.4. Lipid peroxidation

Lipid peroxidation in serum and liver homogenates was measured by thiobarbituric acid reactive substances [17]. To quantify the malondialdehyde bound to the macromolecules serum or homogenate were subjected to alkaline hydrolysis following proposed protocol by Cighetti et al. [18] with the following modifications: in the test tube 100 mg of liver (or 200 μL serum) were homogenized with 1 mL of 1.15% KCl. Then, it was added 3 mL of Milli-Q water and 0.5 mL of 2 M NaOH. After stirring the tubes they were heated at 60 °C for 30 min and then neutralized with 2 M HCl to follow the reaction with thiobarbituric acid. The lipid hydroperoxides were determinated using the ferrous oxidation-xylenol orange (FOX) assay as described by Sodergren et al. [19].

2.5. Protein damage tests

The extent of protein damage in serum and liver has been accessed by carbonyl content and advanced oxidation protein products (AOPP) assay following methodologies of Odetti et al. [20] and Witko-Sarsat et al. [21], respectively.

2.6. Status of antioxidants

The status of antioxidants was accessed by determination of vitamin E (exogenous) and free (mainly glutathione) and total thiols (endogenous). Vitamin E was quantified in liver homogenates by HPLC following methodology described by Arnaud et al. [22] and the obtained values were corrected for lipid content. Thiols (free and total) in liver homogenates were determined by colorimetric assay after 5,5′-dithiobis-(2-nitrobenzoic acid) reaction [23].

2.7. Fat content in liver homogenates

For the quantification of total fat in liver homogenates the method proposed by Bligh and Dyer [24] was used.

2.8. Statistical analysis

Differences between groups were determined by one-way analysis of variance (ANOVA) using a multiple comparison procedure (Tukey). A P value of <0.05 was considered significant. Data are given as mean ± standard deviation of the mean.

3. Results

In Table 1 we note that the groups receiving treatment with thiamine and benfotiamine had higher serum ethanol ($P < 0.05$) than group E + S. The same behavior was observed for liver ethanol concentrations, but with E + BE group reaching values significantly higher ($P < 0.05$) than the E + T group. When we analyze the percentage of hepatic ethanol relative to the initial dose, a significant difference ($P < 0.05$) among the three groups that received ethanol was found, with values of 1.3 ± 1.3%, 2.5 ± 0.9% and 3.6 ± 1.2% for groups E + S, E + T and E + BE, respectively.

The ALT and AST levels (Table 2) were lower ($P < 0.05$) in groups which received treatment with thiamine and benfotiamine.

Table 1
Characterization of the experimental model as the ethanol concentrations in serum and liver.

Parameter	Groups		
	E + S	E + T	E + BE
Serum ethanol (g/L)	1.08 ± 0.85	1.99 ± 0.75*	1.90 ± 0.39*
Hepatic ethanol (mg/g of tissue)	1.32 ± 1.12	3.34 ± 1.38*	5.13 ± 1.83*,**
Hepatic percentage (%) of ethanol in relation to initial dose	1.3 ± 1.3	2.5 ± 0.9*	3.6 ± 1.2*,**

Groups: E + S (Ethanol), E + T (Ethanol treated with thiamine), E + BE (Ethanol treated with benfotiamine).
* $P < 0.05$ relative to E + S group.
** $P < 0.05$ relative to E + T group.