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Effects of acute alcohol consumption and vitamin E co-treatment on oxidative stress parameters in rats tongue

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ABSTRACT

The aim of this study was to evaluate the effects of acute alcohol consumption and vitamin E co-treatment upon oxidative stress parameters in rats tongue. Thirty-eight, *Wistar* rats were separated into five groups (alcohol, alcohol/vitamin E, control, Tween, vitamin E). Alcohol and alcohol vitamin E groups had the standard diet, and 40% alcohol on drinking water. Other groups were fed with the same standard diet and water ad libitum. Vitamin E was given by gavage to vitamin E and alcohol/vitamin E rats twice a week. Alcohol and control groups were subjected to saline gavage and Tween group to 5% Tween 80 solution, the vitamin E vehicle. At day 14, the animals were anesthetized and specimens were obtained from tongue. Lipid peroxidation (TBARS), protein oxidative damage, catalase (CAT) and superoxide dismutase (SOD) activities were quantified. Alcohol group decreased TBARS in relation to control group and alcohol vitamin-treated animals decreased TBARS when compared to Tween and vitamin E groups. SOD activity was lower and CAT activity was higher in animals treated with both alcohol and vitamin E. These results suggest that short-term alcohol consumption decreases lipid peroxidation levels. Alternatively, alcohol/vitamin E group increased CAT, showing the toxicity of this association.

1. Introduction

Oxidative stress has been suggested as playing a central role in many pathways of alcohol-induced damage in a variety of systems, such as liver (Dey and Cederbaum, 2006), central nervous system (Nordman, 1987; Chu et al., 2007) and testes (Nordmann et al., 1990). Alcohol metabolization generates reactive oxygen species (ROS), which may damage macromolecules in the cell, including lipids, proteins and DNA (McDonough, 2003; Wu and Cederbaum, 2003; Das and Vasudevan, 2007).

It has been demonstrated that both acute and chronic alcohol exposure increase ROS production and decrease antioxidant defenses leading to oxidative stress in liver (Halliwell, 1991; Wu and Cederbaum, 2003). Antioxidant enzymes exhibit reduced activity in addition to decreases in the cellular and extracellular content of non-enzymatic antioxidants that should be obtained by diet but are inadequately absorbed by the gastrointestinal system due to alcohol consumption (Wu and Cederbaum, 2003).

Few studies have assessed the short-term effects of alcohol on oral mucosa and has focused only on epithelial permeability (Du et al., 2000; Howie et al., 2001). Biochemical imbalance could be the first signal of alcohol damage.

Therefore, the aim of this study was to assess the acute alcohol intake effects and the possible protective effect of vitamin E co-administration upon oxidative stress parameters and antioxidant enzymes activities after acute alcohol exposure in rats tongue tissues.

2. Materials and methods

2.1. Chemicals

Alcohol was purchased from Cromoline-Química Fina (Diadema, SP, Brazil). Thiobarbituric acid, folin–ciocalteu reagent, 1,1,3,3-tetramethoxypropane, catalase, adrenaline, dinitrophenylhydrazine (DNPH) and vitamin E (α-tocopherol) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Glycine was purchased from Nuclear (Diadema, SP, Brazil). Tween 80 was purchased from Riedel-de-Haën (Seelze, Germany).

Abbreviations: CAT, catalase activity; DNPH, dinitrophenylhydrazine; LSD, least significant difference; ROS, reactive oxygen substances; SOD, superoxide dismutase activity; TBARS, thiobarbituric acid-reactive substances.

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2.2. Animals and treatment

Thirty-eight, 3 months old female Wistar rats *(Rattus norveggicus)*, weighing 190–260 g, were housed in a temperature-controlled room (24 °C ± 1 °C) with 12:12 h reverse light/dark cycle. This experiment was approved by the Research Committee and the Ethics Committee at the Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul (protocol no. 190/05 of Dentistry Graduate Program).

All animals used in the experiments were treated in accordance with The *Guidelines for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes oh Health.

Animals were assigned to one of the following five groups by the stratified weight randomization method:

(1) Control, $n = 6$;
(2) Alcohol, $n = 10$;
(3) Tween 80 (vitamin E solvent), $n = 6$;
(4) Vitamin E + Tween 80, $n = 6$;
(5) Alcohol/vitamin E, $n = 10$.

The control, Tween and vitamin E groups were fed a standard laboratory chow diet for rodents (Nuvilab/CR1, Nuvital Nutrientes LTDA, Colombo, Brazil) and tap water ad libitum. The other experimental groups (Alcohol and alcohol/vitamin E) received the same standard diet but the water was replaced with 40% (vol./vol.) ethyl alcohol throughout experiment, which was available ad libitum as well. The 40% alcohol concentration was chosen because it is the same concentration found in "cachaça", a distilled liquor most frequently consumed by the Brazilian population (Neves et al., 1989). During the first week, the alcohol concentration was increased gradually from 5% to 40%, according to the Table 1 (adapted from McMillen et al., 2005).

Vitamin E (α-tocopherol dissolved in 5% Tween 80 solution) was given orally via gavage (200 mg/kg, twice a week according to Kalender et al. (2004) to alcohol/vitamin E and vitamin E rats. Alcohol and control groups were subjected to saline gavage. Tween treated animals received 5% Tween 80 (Krishnamurthy and Bieri, 1963) solution at the same dose as alcohol/vitamin E and vitamin E animals with the aim to evaluate the effect of the vitamin E vehicle.

The solutions volume (ml) and standard chow diet amount (g) consumption were monitored along study. Animals weight (g) was measured at the beginning and at the end of the experiment, as well as the weight gain (%) during to study was measured.

At day 14, under intra-peritoneal anesthesia using 100 mg/kg ketamine/50 mg/kg xylasine, a specimen was obtained from tongue dorsum by 5-mm biopsy punch, which includes epithelial and connective tissues. This anatomic site was choosen in order to standardize the location and size of the specimen. The tongue biopsy was homogenized in ice-cold phosphate saline buffer (PBS, pH 7.4), sonicated in ice bath (4 cicles of 10 s) and stored at −80 °C for further analyses. It was assessed the Lipid peroxidation (thiobarbituric acid-reactive substances-TBARS), protein oxidative damage (carbonyl groups), catalase (CAT) and superoxide dismutase (SOD) activities.

2.3. Thiobarbituric acid-reactive substances (TBARS)

As an index of lipid peroxidation we used the formation of TBARS during an acid-heating reaction (Valenzuela, 1991). Briefly, 300 μL of sample was mixed with 600 μL of trichloroacetic acid 15% and 500 μL of thiobarbituric acid 0.67%, then heated in a boiling water bath for 20 min. TBARS were determined by absorbance at 532 nm using 1,1,3,3-tetramethoxypropane as an external standard. Results were expressed as malondialdehyde equivalents per milligram of protein.

2.4. Protein carbonyls

Oxidative damage to protein was assessed by determination of carbonyl groups based on their reaction with dinitrophenylhydrazine (DNPH), as described by Levine et al. (1990). Briefly, proteins were precipitated by the addition of 20% trichloroacetic acid to 150 μL of concentrated sample and reacted with DNPH. Then 8 M urea was added to samples and the carbonyl content was determined from absorbance at 370 nm using a molar absorption coefficient of 22,000 M^{-1}. Urea was used instead of guanidine hydrochloride due to higher cost of the former.

2.5. Catalase (CAT) and superoxide dismutase (SOD) activities

In order to determine CAT activity, tissue samples were sonicated in 50 mM phosphate buffer and the resulting suspension was centrifuged at 6200×g for 10 min. The supernatant was used for enzyme assay. CAT activity was measured by the rate of decrease in hydrogen peroxide (10 mM) absorbance at 240 nm (Aebi, 1984). SOD activity was quantified by inhibition of adrenaline self-oxidation absorbance at 480 nm and the tissue samples were not sonicated for this assay (Misra and Fridovich, 1972).

Table 1
Alcohol concentration increase per day in adaptation phase.

Day	1st	2nd	3rd	4th	5th	6th	7th
	5%	10%	15%	20%	25%	30%	40%

2.6. Protein quantification

All of the results were normalized for protein content (Lowry et al., 1951).

2.7. Statistical analysis

Analysis of variance (ANOVA) was used to compare means between groups when data followed normal distribution. Statistical significance was considered when $p < 0.05$. The Tukey test was performed for multiple comparison.

3. Results

3.1. Nutritional parameters

The alcohol treated animals consumed a lower solution volume when compared to control, Tween and vitamin E groups. The same behavior was observed in relation to standard chow diet consumption (Table 2).

Animals weight did no differ from each other at the beginning of experiment ($p = 0.91$), but at the end, the alcohol treated animals' weight were lower (Table 3, $p < 0.05$).

3.2. Lipid peroxidation and protein oxidative damage

Lipid peroxidation levels (TBARS) and protein carbonyls are shown in Fig. 1a and b. Rats subjected to alcohol treatment exhibited lower TBARS levels when compared to control, Tween and vitamin E groups (Fig. 1a, control = 0.130 ± 0.017, alcohol = 0.084 ± 0.036, Tween = 0.148 ± 0.013, vitamin E = 0.160 ± 0.015, alcohol/vitamin E = 0.108 ± 0.031, $p < 0.05$). In addition, alcohol intake associated with vitamin E co-treatment showed lower TBARS levels when compared to vitamin E-treated animals. Protein carbonyls were not significantly different between groups ($p = .554$).

3.3. Superoxide dismutase and catalase activities

SOD and catalase activities are shown in Fig. 2a and b, respectively. SOD activity was higher in Tween group and lower in alcohol/vitamin E group when compared with other groups (Tween = 18.49 ± 12.67, vitamin E = 14.90 ± 5.30, alcohol = 10.55 ± 4.44, control = 8.74 ± 5.25, alcohol/vitamin E = 6.58 ± 3.33, $p < 0.05$). CAT activity was higher in alcohol/vitamin E group when compared to control and vitamin E groups (alcohol/vitamin E = 0.024 ± 0.006, Tween = 0.018 ± 0.006, alcohol = 0.016 ± 0.006, control = 0.014 ± 0.005, vitamin E = 0.011 ± 0.007, $p < 0.05$). SOD/CAT ratio are shown in Fig. 2c. Alcohol/vitamin E animals presented a lower SOD/CAT ratio when compared to vitamin E ones (alcohol/vitamin E, mean rank = 6,57; vitamin E, mean rank = 20,67, $p < 0.05$).

Table 2
Standard chow diet and solutions (water and 40% ethanol solution) consumption.

Group	*n*	Standard chow diet animal/day (g)	Solution animal/day (ml)
Control	6	12.53	37.92
Alcohol	10	6.35	15.15
Tween	6	11.56	38.15
Vitamin E	6	11.73	34.11
Alcohol vitamin E	10	6.48	16.21

Results are expressed as mean.