PROTECTIVE EFFECT OF RESVERATROL ON ETHANOL-INDUCED LIPID PEROXIDATION IN RATS

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(Received 22 July 2005; first review notified 9 August 2005; in revised form 28 October 2005; accepted 29 November 2005; advance access publication 3 March 2006)

Abstract — Aim: Chronic ethanol treatment induces an increase in oxidative stress. As polyphenolic compounds are potent antioxidants, we aimed to examine whether dietary supplementation of resveratrol may attenuate lipid peroxidation, the major end-point of oxidative damage resulting from chronic ethanol administration. Method: Three groups of male Wistar rats were used. The first group served as control and received a daily intraperitoneal injection of 0.9% saline. The second group of rats was daily injected with 35% ethanol at 3 g/kg body weight. The third group was given the same dose of ethanol and supplemented with resveratrol (5 g/kg) in the standard diet. Malondialdehyde (MDA), an indicator of oxidative stress, was measured in the liver, heart, brain, and testis. Results: At the end of a 6 weeks treatment period, MDA levels were significantly increased by 51.5, 53.7, 72.7, and 40.5% in the liver, heart, brain, and testis, respectively. However, when ethanol treated rats were given resveratrol the increase in MDA levels was significantly reduced in all organs to nearly those of control rats. Conclusion: Resveratrol is able to inhibit the ethanol-induced lipid peroxidation and have protective effect against oxidative injury.

INTRODUCTION

According to the so-called ‘French paradox’, moderate red wine consumption could have beneficial health effects (Fremont, 2000). There are increasing evidences that resveratrol (3,4’,5-trihydroxystilbene), a phytalexin mainly found in grapes and red wine is the main active principle implicated in the ‘French paradox’, moderate red wine consumption could have beneficial health effects (Fremont, 2000). There are increasing evidences that resveratrol (3,4′,5-trihydroxystilbene), a phytalexin mainly found in grapes and red wine is the main active principle implicated in the protective activity against alcohol injury. As polyphenolic compounds are potent antioxidants, we aimed to examine whether dietary supplementation of resveratrol may attenuate lipid peroxidation, the major end-point of oxidative damage resulting from chronic ethanol administration.

MATERIALS AND METHODS

Chemicals

Resveratrol was purchased from Selmedica Healthcare (Korea). Butylated hydroxytoluene (BHT) and 2-thiobarbituric acid (TBA) were obtained from Sigma Chemicals Co. (Germany). Absolute ethanol (99.5%) was purchased from Carlo Erba reagent (France). All other chemicals were from Merck (France) and were of the highest grade available.

Animals

Adult male Wistar rats weighing 200–230 g purchased from SIPHAT (Tunis, Tunisia) were used in this study. Before any experience, all animals were kept for 1 week under the same laboratory conditions of temperature (22 ± 2°C), relative humidity (70 ± 4%), and a 12 h light/dark cycle, and received a nutritionally standard diet (SICO, Sfax, Tunisia) and tap water. All experiments were carried out with the approval of the local animal use committee.

Experimental procedure

After a habituation period, rats were divided into three groups of 12 animals each. The first group served as control and received an ip injection of 0.9% (w/v) NaCl. The second group was given a daily ip injection of ethanol (3 g/kg body weight) prepared as a 35% (v/v) solution in 0.9% (w/v) NaCl. The third group was given a daily injection of ethanol and resveratrol that was given as a diet supplement (5 g/kg). Briefly, diet (~15–20 g/animal/day) was freshly prepared from the powder and resveratrol was added just before mixing with a blender. Food intake of control, ethanol, and ethanol + resveratrol rats was daily recorded. At the end of the 6 weeks treatment period, rats were killed by decapitation and liver, heart, brain, and testis were immediately removed, homogenized in a 10 mM ice-cold phosphate buffered saline (PBS), pH 7.4, for lipid peroxidation measurements. Previous studies have been conducted with rats using different ways of ethanol administration and different time of exposure. The alcohol dose of 3 g/kg body weight was chosen because this dose produces moderate toxicity (Ogilvie et al., 1998) and a blood alcohol peak within 15 min of administration via ip route which remain high for at least 3 h (Ogilvie et al., 1997). Alcohol was diluted to 35% to prevent peritoneal irritation.

Lipid peroxidation

The lipid peroxidation product in different tissues was determined by TBARS, expressed as the extent of malondialdehyde...
(MDA) production (Draper and Hadley, 1990). Briefly, tissue homogenates were centrifuged at 10 000 g for 10 min at 4°C to sediment mitochondria and cell debris. The post-mitochondrial samples were suspended in PBS pH 7.4, mixed with BHT–TCA solution (1% w/v BHT dissolved in 20% w/v TCA), and centrifuged at 1000 g for 5 min. Supernatant was then mixed with 0.5 N HCl and 120 mM TBA in 26 mM Tris, and heated in a water bath at 80°C for 10 min. After cooling, the absorbance of the resulting chromophore was determined at 532 nm using a BIORAD UV-Visible spectrophotometer (Smart Spec 3000) and MDA production was determined by using an extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$.

Protein assay

Protein concentrations in the supernatant of each tissue were determined by the method of Bradford (1976) using bovine serum albumin as standard.

Statistical analysis

All results are expressed as mean ± standard deviation. Comparisons between the groups were performed by one-way ANOVA followed by Student t-test. Differences were considered significant at $P < 0.05$.

RESULTS

At the end of the 6 weeks treatment period, body weight of the controls reached 274.42 ± 8.4g compared with 249.45 ± 4.36 g in the ethanol group ($P < 0.01$ vs control) and 267.45 ± 7.81 g in the ethanol + resveratrol group ($P > 0.05$ vs control). Food intake decreased significantly in rats only injected with ethanol and resveratrol supplement restored dietary intake to nearly control levels (data not shown). Resveratrol intake was monitored by total food intake. As at the end of treatment all resveratrol supplemented diet was absorbed, resveratrol intake was considered to be 250 mg/kg body weight/day, which is safe (Crowell et al., 2004). Chronic administration of ethanol led to a significant ($P < 0.01$) increase in lipid peroxidation as indicated by the increase in MDA levels in the liver (Fig. 1), heart (Fig. 2), brain (Fig. 3), and testis (Fig. 4). Ethanol-induced increase in MDA levels were of 51.5% in the liver, 53.7% in the heart, 72% in the brain, and 40.5% in the testis. Resveratrol reduced MDA levels to nearly those measured in control rats. Statistical analysis indicate that resveratrol significantly reduced MDA levels by 38.6, 26.1, 53.1, and 31.3% in the liver, heart, brain, and testis, respectively. It is noteworthy that the strongest inhibitory effect of resveratrol on MDA levels was obtained in the brain.

DISCUSSION

Our data first indicates that chronic alcohol treatment provoked a clear toxicity in rats, as assessed by weight loss and decreased food intake. It also induced oxidative stress as monitored by lipid peroxidation products in several organs. Our data confirmed previous work about ethanol-induced toxicity in liver (Alban et al., 1999), heart (Ribière et al., 1992), brain (Montoliu et al., 1994), and testis (Wright et al., 1991). In the present study, increased lipid peroxidation observed after ethanol treatment might have been enhanced by the high polyunsaturated fatty acid content of the diet, which is known to be particularly vulnerable to ROS.
Our data further indicate that resveratrol, a polyphenol found in red wine, inhibited ethanol-induced lipid peroxidation in rats. Inasmuch as ip injection of ethanol in rats mimicked alcoholism in man, resveratrol appears as a good candidate in the prevention of alcohol-induced injuries in several organs as liver, heart, testis, and brain.

Hepatoprotective effect of resveratrol has been well documented and seems to be related to its antioxidant properties (Cai et al., 2003). Resveratrol also induced a strong decrease in alcohol-induced lipid peroxidation of heart; this could partly explain the cardiovascular beneficial effects of red wine consumption (Bradamante et al., 2004). However, whether resveratrol is the active component, as well as its mode of action, is still controversial (Fantinelli et al., 2005).

Resveratrol also exhibited protective effects on ethanol-induced oxidative injury in testis. Our data are in accordance with previously described beneficial effect of resveratrol on benzoapryrene-induced oxidative DNA damage and apoptosis of sperm (Reval et al., 2001) or more recently with resveratrol protection of testis against injury associated with an ischemia–reperfusion model of oxidative stress (Uguralp et al., 2005).

Nevertheless, the most exciting finding is the neuroprotective effect exerted by resveratrol against alcohol injury obtained in vivo. Our data corroborates those of Sun et al. (1997) who showed that resveratrol protected PC12 cells from peroxidative stress and reduced cell death induced by ethanol. Our data further indicate that resveratrol is able to cross the brain ‘barrier’ (Gilgun-sherhi et al., 2001) and reaches efficient blood level to exert its antioxidant effect in several organs. Recently some investigators have described neuroprotective effect of resveratrol on a mice model of Huntington disease, which seems to be mediated by the sirtuin pathway (Parker et al., 2005).

We are currently investigating the mode of action of resveratrol as well as the putative involvement of detoxifying enzymes, such as superoxide dismutase, glutathione peroxidase, and catalase, which are known to be implicated in several neurodegenerative disorders (LeBowitz et al., 1996). Although preliminary, our data indicate that resveratrol exhibits cardioprotective, hepatoprotective, and neuroprotective properties. However a lot of work will be necessary before the proposal of resveratrol in the treatment of alcoholism as for γ hydroxy-butyrate (Caputo et al., 2005).

REFERENCES


