GENETICS AND CELL METABOLISM

Oxidative Effects of Chronic Ethanol Consumption on the Functions of Heart and Kidney: Folic Acid Supplementation

M.L. Ojeda, M.J. Barrero, F. Nogales, M.L. Murillo and O. Carreras*

Department of Physiology, Faculty of Pharmacy, Seville University, 41012 Sevilla, Spain

*Corresponding author: Department of Physiology and Zoology, Faculty of Pharmacy, Seville University, C/Profesor García Gonzalez nº 2, 41012 Sevilla, Spain. Tel.: +34-954-556-518; Fax: +34-954-233-765; E-mail: olimpia@us.es

(Received 8 March 2012; first review notified 26 March 2012; in revised form 11 April 2012; accepted 16 April 2012)

Abstract — Aims: The principal aim of this study was to investigate the oxidative effects of chronic ethanol consumption on the functions of the heart and the kidney and the possible modification of this effect by folic acid supplementation. Moreover, in order to find whether this oxidative profile affects cardiovascular function, parameters such as heart rate and glomerular filtration rate were also assessed. Methods: Four experimental groups of rats were used: control, ethanol-exposed, control supplemented with folic acid and ethanol-exposed plus folic acid. Ethanol-exposed rats were subjected to a chronic ethanol treatment (2 months), in which the level of alcohol reaches 30% v/v. Diet and ethanol solution were provided ad libitum, and folic acid supplementation was 8 vs. 2 ppm. Energy intake, creatinine clearance and heart rate were determined. Antioxidant enzyme activity and lipid and protein peroxidation of the heart and the kidney were measured by the spectrophotometric method. Results: Ethanol increases heart size and catalase (CAT) activity and decreases lipid peroxidation in heart without changing heart rate. However, in the kidney, ethanol decreases CAT activity, increases lipid peroxidation and decreases glomerular filtration rate. Folic acid supplementation avoids these situations; it does not, however, improve glomerular function. Conclusion: Chronic ethanol consumption has many effects on the antioxidant enzymatic activity of the heart and the kidney, leading to increased renal lipid peroxidation prevented by folic acid supplementation.

INTRODUCTION

Prolonged alcohol abuse causes many toxic and metabolic disorders such as an imbalance between the body’s pro- and antioxidant systems. Alcohol-induced oxidative stress is associated with the metabolism of ethanol, especially with the enzymatic system alcohol dehydrogenase (ADH), by the production of acetaldehyde and NADH, or by contributing to other mechanisms that finally promote enhanced oxidative damage (Ostrowska et al., 2004; Zhang et al., 2004). At low concentrations, most of the alcohol is metabolized by ADH, but when consumed in higher concentrations, such as in a chronic alcohol model, another microsomal ethanol oxidizing system, cytochrome P450IIE1, is induced, leading to the production of free radical species, which cause tissue damage through peroxidation of cell membranes (Teare et al., 1994). Ethanol metabolism takes place primarily in the liver (Lieber, 2004), and in addition to the liver, other organs such as the kidney, brain and heart are also affected by ethanol consumption (Lieber, 1988; Dinu et al., 2005). This makes the kidney and the heart vulnerable to direct damage caused by reactive oxygen species (ROS) (Rodrigo and Rivera, 2002), and both the tissues are deeply related to cardiovascular function.

Alcohol in large amounts is recognized as a cardiovascular risk factor, as it produces alcoholic cardiomyopathy (Kim et al., 2003), promotes the development of hypertension, alters lipid metabolism and the development of atherosclerosis and thrombus formation (Zakhabi, 1997). Some authors suggest that oxidative stress induced by chronic alcohol consumption is implicated in the pathogenesis of alcoholic cardiomyopathy (Edes et al., 1987; Marin-Garcia et al., 1995; Balasubramanjan and Nalini, 2007), increasing lipid peroxidation and decreasing the levels of antioxidants such as GSH (Husain and Somani, 1997; McDonough, 1999; Bau et al., 2007; Seiva et al., 2009). However, these effects differ depending on the focus and the parameters considered in a study.

In the kidney, oxidative stress and ROS-mediated toxicity have been considered as the primary pathways to alcohol-induced injury (Orellana et al., 1998). Moreover, it has been reported that excessive alcohol consumption may increase the risk of kidney damage by enhancing protein and DNA oxidation (Galazyn-Sidorczuk et al., 2009). Therefore, in order to reverse the oxidative ethanol damage caused in the kidney, many authors have recently focused their research upon different treatments methods using antioxidant such as ginger (Shanmugam et al., 2010), ascorbic acid (Mailankot et al., 2009), grape leaf extract (Puri and Suresh, 2008), Hemidesmus indicus R.Br. root (Saravanan and Nalini, 2007), chrysin (Tahir and Sultana, 2011) or methanolic extract of Cnidoscolus aconitifolius (Adaramoye and Aluko, 2011). All these antioxidants protect against oxidative stress in the kidney and improve kidney histoarchitecture. However, only the methanolic extract of C. aconitifolius, at high doses, is known to increase the glomerular filtration rate.

Owing to decrease in the intake and to the fact that alcohol has a negative effect upon its distribution (Fernández-Borrachero et al., 1996; Homann et al., 2000; Villa et al., 2001), folate deficiency is the most common vitamin deficiency in chronic alcoholics (Halsted, 1995). Furthermore, as it has antioxidative properties (Cano et al., 2001), our research group has been using supplementary folic acid diets in ethanol rats in order to prevent oxidative liver damage (Ojeda et al., 2007). In addition, it is known that folic acid treatment reduces plasma homocysteine levels (a risk factor for developing renal insufficiency and cardiovascular disease), which increase after ethanol exposure (Hultberg et al., 1993). Folic acid also improves plasmatic antioxidant capacity in hemodialysis patients (Alves et al., 2007; Tamadon et al., 2011), and its beneficial effects upon the cardiovascular system are not confined only to their participation in homocysteine metabolism since such
effects have also been shown in the absence of hyperhomocysteinemia (Mangoni et al., 2002; Das, 2003; Torrens et al., 2006). It has recently been found that folic acid supplementation, acting as an antioxidant, inhibits nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-mediated superoxide anion production in the kidney (Hwang et al., 2011).

For these reasons, the aim of this work was to study whether a folic acid-supplemented diet administered to chronic-ethanol rats could change the oxidative profile of the heart and the kidney and whether these modifications are related to the function of the kidney and heart in rats.

MATERIALS AND METHODS

Animals

This experimental work was carried out in conformance to The Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, DC, 1996) and was approved by the Committee of Animal Use for Research at Seville University, Spain (RD 1201/2005 of 10 October 2005). Forty male Wistar rats weighing ~250–300 g. (Centre of Animal Production and Experimentation, Vice-Rector’s Office for Scientific Research, University of Seville) were housed in stainless-steel cages (two animals/cage) in a well-ventilated temperature-controlled room, (22–23°C) with 12 h/12 h light/dark cycles. The rats were randomly distributed into four groups of 10 rats each and given different treatments for 8 weeks: control group (C)—water and rat basal diet; alcohol group (A)—ethanol and rat basal diet; alcohol + folic acid group (AF)—ethanol and folic acid diet and control + folic acid group (CF)—water and folic acid diet. Diet, water and ethanol solution were provided ad libitum. The experimental period lasted 8 weeks and was divided into two 4-week phases: alcoholic induction and chronic alcoholism, respectively. At the end of the experimental period, the rats were made to starve for 12 h in metabolic cages in order to collect their urine during starvation. Diets were prepared according to Institute of Laboratory Animal Resources (ILAR, 1979), the composition of which was as follows (g/kg of diet): Casein: 200; Sucrose granulated: 510; Cornstarch: 140; Fiber, cellulose: 50; Corn oil: 50; AIN-76 mineral mix: 35; AIN-76 vitamin mix: 10; Choline bitartrate: 2 and DL-methionine: 3. The diet ingredients were mixed and homogenized in a double-cone blender (Rest Haan, Germany), and offered to animals as pellets. The folic acid-supplemented diets contained 8 ppm of folic acid (Acofarma, Barcelona, Spain), while the non-supplemented one contained 2 ppm of folic acid.

Ethanol treatment

Ethanol treatment has been widely used by our research group (Carreras et al., 1992). Alcohol-fed animals (A and AF) were started on tap water that contained 5% v/v alcohol in the first week (initial phase). The alcohol concentration was increased to 10% in the second week, 15% in the third week, 20% in the fourth week (induction phase) and, finally, a 30% solution was maintained for 1 month (chronic alcohol phase). The control groups drank water during the 8-week period. During the whole experimental period, the intake of both solids and liquids by the rats was measured and the animals were weighed using analytical scales accurate to within 0.1 g (COBOS D-2000-SX).

Energy consumption

The total amount of kcal consumed by the animals was estimated by measuring the amount of daily food intake and multiplying it by 2.9 cal. This result was added to the amount of ethanol consumed per day by the rats multiplied by 7.1 kcal. We calculate the amount of ethanol consumed per day by the rats, using the ‘Table for Determining Grams Values of Ethanol Solutions’ devised by Veale and Myers (1968). The amounts of ethanol consumed were A: 3.53 g/kg per day and AF: 3.89 g/kg per day—there was no statistical difference.

Samples

Kidney and heart samples: at the end of the experimental period, the rats were weighed before being anesthetized with intraperitoneal 28% w/v urethane (Sigma-Aldrich, St Louis, USA) at 0.5 ml/100 g of body weight. The organs were extracted in ice-cold physiological saline solution, and all the blood was removed and tissues were debrided of adipose and connective tissue before weighing. The samples were immediately stored at ~80°C prior to the determination of biochemical parameters. Serum samples: blood was collected by the rat tail cut method at the end of the experimental period and the serum prepared by centrifugation (4°C for 10 min at 800 g). Urine samples: urine samples were collected by placing the rats in individual metabolic cages for 24 h. Urine and serum samples were collected at the end of the first, fourth and eighth weeks of treatment.

Estimation of biochemical parameters: oxidative balance

In order to measure the activity of antioxidant enzymes as well as the oxidation of lipids and protein, kidney and heart tissue samples were homogenized (2500 rpm/min for 1 min, 1:4 w/v) (Pobel 245432, Spain) in a sucrose buffer (15 mM TRIS/HCl, pH 7.4, 250 mM sucrose, 1 mM ethylenediaminetetraacetic acid and 1 mM dithiothreitol) and in a potassium phosphate buffer 10 mM (K2HPO4/KH2PO4 pH 7), respectively, in an ice bath. The homogenate was centrifuged at 3000 rpm for 10 min at 4°C. The resulting supernatant was employed for a biochemical assay of the kidney, and the heart supernatant was again centrifuged at 15,000 rpm for 15 min at 4°C, which was used for a biochemical assay of the heart. The degree of lipid peroxidation in the supernatant was evaluated by a colorimetric reaction with thiobarbituric acid (TBA) as described by Draper and Hadley (1990). The malonaldehyde is the end-product of fatty acid peroxidation that reacts with TBA to form a pink complex, which was determined at 535 nm. The results were expressed as mol/mg protein. Protein oxidation was measured by a method based on the spectrophotometric detection of the reaction of 2,4-dinitrophenyldrazine (DNPH) with protein carbonyl (PC) to form protein hydrazones (Reznick and Packer, 1994). The level of PC was calculated at the maximum absorbance (366 nm) and the results were expressed as nmol/mg protein. The activity of superoxide dismutase (SOD) was determined by Fridovich (1985)’s method. This is based on the ability of SOD to inhibit the cytochrome-c reduction by 7.1 kcal.
induced by the xanthine-xanthine oxidase system. SOD activity is expressed as U/mg protein. One enzyme unit is defined as the quantity of SOD required to produce 50% inhibition of the cytochrome-c reduction. Glutathione reductase (GR) activity was measured by NADPH-coupled assay using GSH as the substrate (Worthington and Rosemeyer, 1974). Specific activity was expressed as mU/mg protein, where 1 mU is equal to the nanomoles of NADPH oxidized/min. Catalase (CAT) activity was determined using H2O2 as the substrate by the method of Beers and Sizer (1952). The disappearance of H2O2 was followed spectrophotometrically at 240 nm. The enzymatic activity of CAT was expressed as U/mg protein, where one unit of activity is equal to the amount of micromoles of H2O2 degraded/min. The activity of glutathione peroxidase (GPx) was determined by NADPH-coupled assay according to the method of Lawrence and Burk (1976), and this enzyme catalyses the oxidation of glutathione by hydrogen peroxide. The oxidation of NADPH was followed spectrophotometrically at 340 nm. Specific activity was expressed as mU/mg protein, where 1 mU is equal to the number of nanomoles of NADPH oxidized/min. The protein content of the individual samples was determined by the method of Lowry et al. (1951), using bovine serum albumin as the standard.

Creatinine clearance: serum and urine levels

The creatinine level in both serum and urine was determined by colorimetry, using a commercial kit (BioSystems ref. 11802, Barcelona, Spain). For urine, the sample was previously diluted with bi-distilled water to a ratio of 1/50. Creatinine clearance (CLcr) was calculated using the standard clearance formula: CL = U × V/P, where U is the creatinine level in urine, V the volume of urine collected in 24 h and P the level of creatinine in plasma.

Cardiac frequency

This was monitored using the indirect tail occlusion method employed by Gómez-Amores et al. (2007). Measurements were taken at the end of the first, fourth and eighth weeks of treatment, using a NIPREM 645 (CIBERTEC, Spain) pressure meter. The signals collected were treated with an IT support via a data acquisition system coupled to a pressure meter. Each animal was measured 4–5 times successively in order to calculate the arithmetical mean, which was used in the experiment.

Statistical treatment

The results were expressed as the mean ± SEM, where n is the number of samples analyzed. In order to compare the different variables under study in the different experimental groups, the data were analyzed using the one-way analysis of variance test followed by Tukey–Kramer tests with the GraphPad InStat 3 statistical software. Values of P < 0.05 were considered to be statistically significant.

RESULTS

Nutritional status

Table 1 shows that the folic-acid-supplemented rats consumed a greater amount of folic acid than the non-supplemented ones (P < 0.001). In this context, it can be seen that ethanol consumption decreases the intake of this vitamin (A vs. C P < 0.5, AF vs. CF P < 0.001). Despite the fact that the total kcal intake is similar throughout the four groups, the solid kcal intake is higher (P < 0.001) in the control groups (C and CF) than in the alcohol-exposed rats (A and AF). However, the liquid kcal intake is higher in the ethanol rats (Table 1). Body weight is higher in the control rats (C and CF) than in their ethanol counterparts (P < 0.001).

Heart and kidney weights

Although when the heart weight was measured, no differences were found among the groups, the ethanol-fed rats had a significantly higher relative heart weight [organosomatic index (IOS)] than the control group (P < 0.05). The supplemented diet prevented this increase (Table 1). There were no differences in the kidney weight or the relative kidney weight among the groups (Table 1).

Heart antioxidant enzymes activities

As shown in Fig. 1, GPx and SOD activities are similar in all the groups; however, GR activity increases after the folic acid supplementation (C vs. CF P < 0.01 and A vs. AF P < 0.05), and CAT activity is significantly increased in the A

Table 1. Nutritional parameters

<table>
<thead>
<tr>
<th></th>
<th>C: n = 10</th>
<th>A: n = 10</th>
<th>AF: n = 10</th>
<th>CF: n = 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic acid intake (µg/rat/day)</td>
<td>40.5 ± 1.8</td>
<td>22.8 ± 2.5**</td>
<td>103.2 ± 3.4***</td>
<td>181.6 ± 3.3****</td>
</tr>
<tr>
<td>Fluid kcal intake (kcal/rat/day)</td>
<td>86.65 ± 2.5</td>
<td>55.61 ± 2.5***</td>
<td>52.21 ± 2.2***</td>
<td>91.30 ± 3.2</td>
</tr>
<tr>
<td>Total kcal intake (kcal/rat/day)</td>
<td>—</td>
<td>24.9 ± 2.3</td>
<td>28.19 ± 2.5</td>
<td>—</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>426.6 ± 9.3</td>
<td>328.7 ± 9.6***</td>
<td>328.5 ± 9.8***</td>
<td>435.4 ± 9.7</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>1.17 ± 0.03</td>
<td>1.07 ± 0.02</td>
<td>1.02 ± 0.02</td>
<td>1.16 ± 0.03</td>
</tr>
<tr>
<td>IOS heart</td>
<td>0.5 ± 0.005</td>
<td>0.34 ± 0.009**</td>
<td>0.3 ± 0.01</td>
<td>0.31 ± 0.005</td>
</tr>
<tr>
<td>Kidneys weight (g)</td>
<td>2.79 ± 0.2</td>
<td>2.28 ± 0.19</td>
<td>2.30 ± 0.21</td>
<td>2.81 ± 0.2</td>
</tr>
<tr>
<td>IOS kidney</td>
<td>0.64 ± 0.03</td>
<td>0.69 ± 0.03</td>
<td>0.7 ± 0.03</td>
<td>0.65 ± 0.03</td>
</tr>
</tbody>
</table>

Folic acid intake and solid, liquid and total kcal intake during the experimental procedure (8 weeks). Body, renal and heart weights at the end of the experimental procedure.

Results are mean ± SEM of 10 animals in each group. C, control group; A, alcohol group; AF, alcohol + folic acid group; CF, control + folic acid group; IOS, organosomatic index. Statistic significance: A vs. C: ***P < 0.001,*P < 0.05; AF vs. A: **P < 0.01, *P < 0.05; AF vs. CF: ***P < 0.001; C vs. CF: ***P < 0.001.
groups with respect to the C and the AF groups ($P < 0.01$).

In Fig. 2, we can observe that protein oxidation is similar in the four groups. However, lipid oxidation decreases significantly in the A rats with respect to the C ($P < 0.01$) and the AF ($P < 0.05$) ones. Comparing the activities of these enzymes using the GR/GPx and CAT/SOD ratios (Fig. 5A and B), we observe no differences in the GR/GPx ratio among the groups, but we find a significant increase in the CAT/SOD ratio in the ethanol rats (A) with respect to the C and the AF ones ($P < 0.01$).

Kidney antioxidant enzyme activities

Figure 3 shows that GPx kidney activity is similar in the four experimental groups, GR activity increases after folic acid supplementation in the alcohol and the control rats ($P < 0.05$), SOD activity is similar in the four groups and that CAT activity decreases significantly in the ethanol-fed groups with respect to the C ($P < 0.05$) and the AF ($P < 0.01$) groups. In Fig. 4, we can observe that protein oxidation is similar in the four groups. However, lipid oxidation is higher in the ethanol-fed rats with respect to the
control and the ethanol-folic acid ones \((P < 0.05)\). When analyzing the GR/GPx and CAT/SOD ratios (Fig. 5C and D), we observe no differences in the first ratio among the groups, but we find a significant decrease in the CAT/SOD ratio in the ethanol rats \((A)\) with respect to the C and the AF ones \((P < 0.01)\).

**Cardiac frequency**

Cardiac frequency was not significantly affected by alcohol intake or folic acid intake during the whole experimental period (Fig. 6).

**Creatinine clearance**

In the first and the fourth weeks of the experimental procedure, no differences in creatinine clearance were found among the groups. However, at the end of the experimental period (8th week), the alcohol-exposed rats \((A\) and \(AF)\) had lower \(CL_{cr}\) values than the controls \((P < 0.001)\) (Fig. 7).

**DISCUSSION**

It is known that when alcohol consumption is moderate \((16\%\) of total dietary energy), the energy supplied by ethanol results in a slight increase in total energy consumption. In contrast, as alcohol intake increased, there was a decrease in the percentage of energy derived from protein, fats and carbohydrates, and the nutritional quality of the diet declined with body weight \((Hillers and Massey, 1985)\). As our ethanol-treated rats received 30–35\% kcal from diet, our results were completely in agreement with those obtained by the aforementioned authors.

**Studies of the heart**

The increase in relative heart weight found in the alcohol group implies that after chronic ethanol consumption, these animals develop cardiomegaly. This fact has been widely described in the literature, alcoholic cardiomyopathy being regarded as a pathology associated with chronic ethanol intake \((Iacovoni et al., 2010)\). It is defined as a heart muscle disease that occurs initially in an asymptomatic stage and becomes symptomatic only when the disease is well developed, but little is known about its etiology and treatment. However, the folic-acid-supplemented ethanol rats did not show an increase in relative heart weight, despite the fact that their food and alcohol intakes were similar to those of the ethanol rats and that they had similar body weight. Therefore, this increase is not directly related to a decrease in body weight. In this context, \(Fahimi et al. (1979)\) and \(Panchenko et al. (1987)\) have found that, following ethanol consumption, there is an increase in the size and number of peroxisomes of the heart, where the enzyme CAT (which is also stimulated) is located. They associate this peroxisome increase with increase in heart size. These investigations are in agreement with our results. We have found an increase in heart CAT activity after ethanol consumption, an activity which decreases when ethanol is consumed together with folic acid.
The detoxification pathway is the result of multiple enzymes, with SOD catalyzing the first step and then CAT and glutathione peroxidase removing the hydrogen peroxide generated. In this study it can be observed that in the heart SOD activity was not affected by alcohol or folic acid intake. However, CAT activity increased in the ethanol-exposed rats but not in those supplemented with folic acid. This enzyme catalyzes the reduction of H$_2$O$_2$, generating H$_2$O and O$_2$, but to a lesser extent, it is also involved in the oxidative metabolism of alcohol. In the heart, unlike the liver, a low ADH presence is described and the existence of the microsomal ethanol oxidizing system is unknown (Fahimi et al., 1979). As we mentioned earlier, an increase in the level of peroxisomes with CAT activity after ethanol consumption (Herzog and Fahimi, 1976; Hicks and Fahimi, 1977) has been reported in cardiac muscle, indicating that CAT plays a more prominent role in the metabolism of alcohol in the heart than the ADH system (Fahimi et al., 1979; Antonenkov and Panchesko, 1986; Panchesko et al., 1987). Therefore, when rats consume a greater amount of ethanol, the activity of this enzyme increases. CAT activity is highly related to SOD activity, because it acts by removing the hydrogen peroxide that SOD activity generates. When comparing the ratio between these two antioxidant enzymes, we observe that ethanol exposure increases this value and that folic acid supplementation concomitant with ethanol has similar CAT/SOD balances as controls. These results indicate that the hearts of ethanol-exposed animals have a greater ability to avoid the accumulation of H$_2$O$_2$, and therefore, probably a greater resistance to oxidative damage. In fact, when lipid and protein oxidation in the heart were compared, the ethanol-fed rats presented lower lipid oxidation than the rest of the groups. It seems that the heart responds to chronic alcohol consumption by increasing its efficiency to transform the H$_2$O$_2$ via CAT. However, this effect disappeared when folic acid was administered. This evidence leads us to postulate that, by some unknown mechanism, folic acid supplementation acts in the heart by reducing the need to increase CAT activity after ethanol exposure. This might be due to the fact that this nutrient affects the antioxidant defense systems involved in the balance of glutathione, and therefore in the balance of GR/GPx, which also prevents the accumulation of H$_2$O$_2$. It is known that folic acid supplementation to ethanol-exposed pups increases hepatic GSH levels related to the homocysteine methionine cycle (Ojeda et al., 2009). This might be, as Davis and Uthus (2003) defend, due to the fact that folic acid increases GSH regeneration via homocysteine transsulfuration. In contrast, it is known that ethanol increases Hcys production and decreases GSH levels (Stickel et al., 2000).

GR activity is intimately related to GPx activity because it reduces the glutathione disulfide (GSSG) created by GPx to the antioxidant sulfhydryl form (GSSH), which is required for GPx activity. We have found that despite the fact that ethanol consumption does not affect these two enzyme activities, folic acid supplementation increases GR activity leading to an increase in GSSH generation. This increase in the endogenous antioxidant GSH could be limiting the stimulation of CAT activity.

Although it has been reported that the acute and chronic consumption of ethanol can affect cardiac function by affecting the oxidative balance of the heart decreasing GPx and SOD activities in dogs (Jing et al., 2012), there is controversy in the literature and there is no a clear demonstration that oxidative damage causes cardiomyopathy induced by ethanol consumption (McDonough, 1999). In this context, some researchers do not relate ethanol oxidation to cardiomyopathy (Fahimi et al., 1979; Antonenkov and Panchesko, 1986; Edes et al., 1987; Panchesko et al., 1987), while others do (Ashakumary and Vijayammal, 1996; Husain and Somani, 1997; Yildiz et al., 2000; Zhang et al., 2004; Balasubramaniyan and Nalini, 2007). In our opinion, both the dose of ethanol administered and the duration of treatment affect the antioxidant enzymes’ activity and its effects (Husain and Somani, 1997), showing a dose-dependent effect—even for the same tissue (Scott et al., 2000). According to that, all studies based on administration of alcohol to animals orally as part of a liquid diet (Fahimi et al., 1979), in a semisolid diet (Antonenkov and Panchesko, 1986; Panchesko et al., 1987) or diluted in drinking water (Edes et al., 1987) along with food, have shown similar results. This suggests that oral administration of ethanol along with food affects the assessment of oxidation in heart. Maybe the first metabolic action of ADH in the stomach, and the intestinal alcohol absorption, could be related to the amelioration of oxidative heart damage caused when alcohol is administered by an ad libitum model of food and drink. Recently, Fogle et al. (2010) have reported that one of the main mechanisms of the pathogenesis of alcoholic cardiomyopathy involves modifications in protein expression secondary to inhibition of protein synthesis, and the cause of these effects remains to be investigated. Cheng et al. (2006) have concluded that chronic alcohol consumption produces rennin–angiotensin system activation followed by progressive cardiac dysfunction, and that the cardiac dysfunction is prevented by angiotensin II type 1 receptor blockade.

Despite the cardiomegaly observed in ethanol rats, cardiac frequency is not affected by alcohol intake, in spite of the fact that the dose consumed was 30%, these data agree with those obtained by Resstel et al. (2006). However, in previous studies with similar rats, we found that ethanol increases arterial blood pressure. In this situation, a dietary supplementation with folic acid reduces arterial blood pressure by reducing the levels of aldosterone in blood, since it favors renal elimination of the hormone (Barrero et al., 2012). For this reason, consumption of alcohol in large amounts is recognized as a cardiovascular risk factor, as it produces alcoholic cardiomyopathy (Kim et al., 2003) promotes the development of hypertension, alters lipid metabolism and favors the development of atherosclerosis and thrombus formation (Zakhar, 1997). However, in this study, it did not increase heart lipid peroxidation or heart rate, so we cannot confirm that the deleterious effects of ethanol on the functions heart are related to lipid peroxidation, despite its being related to the antioxidant enzyme CAT.

Studies of kidney

A proper function of the cardiovascular system depends not only on the condition of the heart and blood vessels but also on other organs such as the kidney.

Macroscopically, we did not found modifications in the kidney or relative kidney weight after 8 weeks of ethanol consumption, probably because, as reported by Dina et al.
and functional studies, that chronic alcohol feeding should damage, as CAT limits the accumulation of H2O2 and avoids CAT activities found in the kidney and the heart. In this lactate dehydrogenase and malate dehydrogenase (Nechifor the kidney is the result of an increase in the activity of ADH, exposure on the liver is mainly due to the upregulation of the microsomal ethanol oxidizing system (Rodrigo and Rivera, 2002), the effect on the heart is due to an increase in peroxisomes CAT activity (Fahimi et al., 1979), and the effect on the kidney is the result of an increase in the activity of ADH, lactate dehydrogenase and malate dehydrogenase (Nechifor and Dinu, 2011). This might be the reason for the different CAT activities found in the kidney and the heart. In this context, Kobayashi et al. (2005) have found that acatalasemia exacerbates renal oxidant tissue injury, suggesting a central role for CAT in defending against oxidant renal damage, as CAT limits the accumulation of H2O2 and avoids the Fenton reaction—generation of the highly injurious hydroxyl radical.

After folic acid supplementation, lipid peroxidation in the kidney decreased to control values, acting as an antioxidant in this tissue, not only because it increases CAT activity but also because it increases GR activity, which reduces GSSG to GSH. This increase in GR activity is of great importance because it is well known that in kidney cells the concentration of GSH is lower than in other tissues. Furthermore, the kidney uses methionine as a cysteine precursor for GSH biosynthesis to a lesser extent than other tissues (Adaramoye and Aluko, 2011). At this point, folic acid supplementation is efficient, probably because of the increase in CAT activity and increase in GSH levels, which is in accordance with the findings of Adaramoye and Aluko (2011), who concluded that another antioxidant, methanolic extract of C. aconitifolius leaf, attenuates the ethanol-induced lipid peroxidation in the kidney by increasing CAT, SOD and GSH levels. However, in this study, the GR/GPx ratio is not affected by ethanol exposure. Despite the fact that there is no report in the literature on the relationship between ethanol, folic acid and Se, there is evidence of their implication in the metabolism of hepatic methionine.

To estimate the glomerular filtration rate (GFR) and to evaluate renal function, we also analyzed renal creatinine clearance (CLcr). When the experimental period was over, the clearance values of the two alcohol groups had decreased, indicating an association between ethanol intake and a lower renal filtration rate. This reduction in creatinine clearance is nearly 50%, similar to that found by Van Thiel et al. (1977), who proposed, after in-depth morphological and functional studies, that chronic alcohol feeding should be considered nephrotoxic. Therefore, this decrease in GFR after ethanol consumption was expected, because glomerulus is considered to be more sensitive to oxidative injuries than other nephron segments (Gwinner et al., 1998). This fact is associated with a glomerular LDL accumulation (Lee and Kim, 1998), which activates the apoptosis pathway of endothelial and mesangial cells (Wheeler et al., 1994). Moreover, oxidative stress might also be involved in other inflammatory glomerular lesions caused by a series of mediators, including cytokines and chemokines (Rodrigo and Rivera, 2002). Despite the fact that folic acid has antioxidant properties it does not restore this filtration rate. It is possible that the antioxidant activity reached is not enough to avoid the renal dysfunction or that there are other mechanisms that cause this situation. In this context, the GFR is affected only at week 8 of treatment, showing that this effect is associated with long-term chronic alcohol intake, which fact is related to the effect of alcohol upon arterial pressure, describing a J-shaped curve (Tomson and Lip, 2006). Therefore the duration and the quantity of alcohol ingested would appear to be a key factor in blood pressure and GFR homeostasis (Barrero et al., 2012). In fact, alcohol may lead to kidney disease by directly damaging the kidney (Cecchin and Marchi, 1996), or by elevating blood pressure (Parekh and Klag, 2001) by increasing renal blood flux, glomerular hydrostatic pressure and glomerular filtration velocity. To avoid this situation, the autoregulatory mechanism mediated by the cells of the macula dense provokes a vasoconstriction of the afferent arteriole when they detect high levels of Na+ in the tubular liquid, decreasing the glomerular filtration velocity. After ethanol consumption, there is a direct stimulation of the sympathetic system that stimulates the renin-angiotensin-aldosterone system leading to a general vasoconstriction and an increase of Na+ renal resorption (Tomson and Lip, 2006). This effect of ethanol causes an increase in blood pressure and a deeply high serum aldosterone level (Barrero et al., 2012). It could be the case that the increase in blood pressure combined with the high levels of Na+ in renal tubule stimulates the cells of macula dense greatly, leading to a high arteriole afferent vasoconstriction and a high reduction of the glomerular filtration velocity.

CONCLUSION

In conclusion, chronic ethanol consumption has different effects on the antioxidant enzymatic activity of the heart and the kidney, mainly on CAT activity, leading to increased renal lipid peroxidation prevented by folic acid supplementation. We recognize chronic ethanol consumption as a cardiovascular risk factor, because it promotes cardiomegaly and decreases glomerular filtration rate. In this study alcohol did not increase heart lipid peroxidation or heart rate, so we cannot confirm that the deleterious effects of ethanol on the heart are related to lipid peroxidation, despite its being related to the antioxidant enzyme, CAT. Folic acid supplementation decreases CAT activity and consequently leads to cardiomegaly, and decreases kidney lipid peroxidation, but it does not avoid the renal dysfunction. Owing to the fact that folic acid decreases blood pressure in chronic ethanol rats, we opine that it is a good supplement for chronic ethanol rats with cardiovascular risk.

Dinu D, Nechifor MT, Movileanu L. (2005) Ethanol-induced altera-


Halsted CH. (1995) Alcohol and folate interactions: clinical impli-

Herzog V, Fahimi HD. (1976) Identification of peroxisomes (micro-


Hicks L, Fahimi HD. (1977) Peroxisomes (microbodies) in the myocardium of rodents and primates. A comparative ultrastru-


Hwang SY, Siow YL, Au-Yeung KK et al. (2011) Folic acid sup-


Iacovoni A, De Maria R, Gavazzi A. (2010) Alcoholic cardiomyop-


Kobayashi M, Sugiyama H, Wang DH et al. (2005) Catalase deficien-


Lieber CS. (2004) The discovery of the microsomal ethanol oxidiz-


Lowry OH, Rosebrough NJ, Farr AL et al. (1951) Protein measure-


Mailankot M, Jayalekshmi H, Chakrabarti A et al. (2007) Alcoholic cardiomyopathy in adult turkeys: effects on myocardial antioxi-


McDonough KH. (1999) The role of alcohol in the oxidant antioxi-
