ELSEVIER

Alcohol 45 (2011) 381–391

ALCOHOL

Moderate ethanol ingestion, redox status, and cardiovascular system in the rat

Carmen Gonzalez Martin[a,b,c], Victoria Vega Agapito[a,b,c], Ana Obeso[a,b,c], Jesus Prieto-Lloret[a,b,c], Rosa Bustamante[a,b,c], Javier Castañeda[a,b,c], Teresa Agapito[a,b,c], Constancio Gonzalez[a,b,c,*]

[a]*Departamento de Bioquímica y Biología Molecular y Fisiología, Universidad de Valladolid, Facultad de Medicina, Valladolid, Spain*
[b]*Instituto de Biología y Genética Molecular, Consejo Superior de Investigaciones Científicas (CSIC), Valladolid, Spain*
[c]*Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Valladolid, Spain*

Received 19 April 2010; received in revised form 28 July 2010; accepted 7 August 2010

Abstract

Moderate intake of alcoholic beverages decreases the incidence of cardiovascular pathologies, but it is in dispute if cardioprotective effects are due to ethanol, to polyphenolic compounds present in beverages or to a combination of both. In humans, effects of high, moderate, and low doses of alcoholic beverages are widely studied, but effects of pure alcohol remain unclear. On the other hand, experiments with laboratory animals are centered on high toxicological doses of ethanol but not on low doses. In the present study, we have aimed to mimic in the rat the pattern of alcohol intake in Mediterranean population. Alcohol ingestion is spread along the day and not always related to solid food consumption. We tried to define the beneficial and harmful effects of pure ethanol ingestion without polyphenol's influence. Experimental rats were given 1% ethanol in their drinking water for 30 days, resulting in a daily ingestion of 0.27 mL of ethanol/rat/d. Ethanol ingestion did not cause deleterious effects on the general status of the animals, but it decreased cholesterol, triglycerides, and catecholamine stores' rate of utilization in peripheral sympathetic system. Moreover, ethanol lowered pulmonary arterial pressure and did not alter systemic arterial pressure. In the liver, the reduced glutathione/oxidized glutathione ratio was augmented and lipid peroxide, superoxide dismutase, and glutathione peroxidase activities were decreased. However, catalase activity was unaltered. Liver cytochrome P4502E1 distribution and protein level and activity were unchanged by ethanol ingestion. Data indicate a lack of harmful effects and underscore a set of potentially beneficial effects of this dose of ethanol.

Keywords: Oxygen reactive species (ROS); Antioxidant enzymes; Cardiovascular system; Pulmonary arterial hypertension; Cholesterol; Cytochrome P4502E1

Introduction

Ethanol is an important component of wine and has constituted as an important voluntary component of human diet for thousands of years, and yet, in spite of the stirring of the field produced by the formulation of the French paradox (Ferrieres, 2004), there are not conclusive doctrines on the effects of moderate alcohol ingestion in human health in the epidemiological and observational studies (Blackhurst and Marais, 2005; Goldberg et al., 2003; Govoni et al., 1994). This status reveals that there are many variables associated to human drinking (e.g., amount, type of beverage, form or cadence of ethanol ingestion, and other lifestyle and uncontrolled psychosocial variables). The margin of ethanol ingestion considered in studies is very wide, with moderate ingestion having an upper limit of around 40 g/d for adult men (Govoni et al., 1994; Hines et al., 2001) and a high toxicological ingestion with undefined limits; some authors had tried to elucidate what might be considered as physiological or toxicological effects of ethanol (Bonnefont-Rousselot et al., 2001; McDonough, 2003). The type of beverage is another variable of interest. Thus, in epidemiological studies, the beneficial effects of moderate ethanol ingestion are usually (De Gaetano et al., 2003; Di Castelnuovo et al., 2002; Gronbaek et al., 1995), but not always (Klatsky et al., 1997; Mukamal et al., 2003; Rimm et al., 1996), best evidenced if the beverage is rich in polyphenols and other antioxidant compounds (e.g., red wine and dark beer; see Li and Mukamal, 2004). However, the poor bioavailability and metabolism of the antioxidants present in beverages renders difficulty in the acceptance of physiologically relevant effects (Li and Mukamal, 2004; Williamson and Manach, 2005). In this regard, Rimm et al. (1996) concluded that

* Corresponding author. Departamento de Bioquímica y Biología Molecular y Fisiología, Facultad de Medicina, Universidad de Valladolid, 47005 Valladolid, Spain. Tel.: +34-983-42-30-89; fax: +34-983-42-35-88.
E-mail address: constanc@ibgm.uva.es (C. Gonzalez).

0741-8329/$ - see front matter
doi: 10.1016/j.alcohol.2010.08.003

alcohol consumption can be linked directly to an individual's risk of coronary heart disease, providing strong evidence that all alcoholic drinks are linked with lower risk of coronary heart disease. Similar conclusions have been attained by Klatsky et al. (1997) and Li and Mukamal (2004). The cadence of drinking adds complexity in evaluating the effects of ethanol in epidemiological studies. With the same amount of ingestion per week, there are clear differences; beneficial effects are best evidenced in steady daily moderate drinkers versus weekend-oriented drinkers (Ferrieres, 2004; Govoni et al., 1994). Furthermore, it is conceivable that for the same amount of ethanol ingested per diem, peak plasma ethanol concentrations must be quite different in meals-related drinkers (Mediterranean cultures) versus happy hour-type drinkers.

However, experimental studies in animal models have been oriented toward the high toxicological doses (McDonough, 2003), with very few studies using physiologically relevant doses of ethanol and with limited parameters monitored (Arola et al., 1997).

Our aim in the present study has been to administer ethanol to rats in the amount and cadence that can correspond to the most common drinking behavior in Mediterranean countries: two to three glasses of wine with 11% of ethanol distributed around the day (200–300 mL of wine/d or 18–27 g of ethanol/d). To achieve this level of ingestion and a comparable drinking cadence in rats, we determined in pilot experiments that adding 1% ethanol (vol/vol) to the drinking water resulted in ad libitum daily intake of 0.22 g of ethanol/rat/d. As it is well known, rats metabolize ethanol threefold faster than human, 300 mg/kg body weight/h in rats (Mikata et al., 1963) versus 100 mg/kg body weight/h in humans (Wartburg, 1967). Consequently, this 1% ethanol administration in rats is equivalent to 18 g in a standard human male, a value well within the recommendation for cardiovascular protection (Costanzo et al., 2010). Our data evidenced a total absence of harmful effects and not only that, an amelioration of certain parameters that lend support to the notion that moderate and spaced alcohol drinking might have beneficial health effects.

Materials and methods

Experimental animals

Male adult Wistar rats had free access to standard rat solid diet (A04; Panlab SL, Barcelona, Spain) and drinking water until sacrifice. Rats were weighed at the start and at the end of the experiments. For a period of 30 days, the rats received drinking water with 1% ethanol (experimental group) or regular tap water (control group); both beverages were freshly prepared once a day with a daily measurement of water ingestion. We did not use nasogastric intubation because it is not a voluntary consumption of drink. Experimental protocols were performed in anesthetized rats (we used sodium pentobarbital 60 mg/kg body weight or ketamine 133 mg/kg in the experiments of blood pressure recordings). The Institutional Committee of the University of Valladolid for Animal Care and Use approved the protocols.

Blood and tissue removal

Blood was obtained by direct cardiac puncture after an ample thoracotomy. The plasma was obtained after 2,000*g*, 15 min centrifugation of whole blood at 4°C and was assayed immediately or stored at −80°C.

Livers were quickly excised from rats, washed in ice-cold saline, immediately immersed in liquid nitrogen to freeze, and stored at −80°C.

Plasma ethanol levels

Ethanol analysis was performed in plasma obtained from EDTA collected blood using the enzymatic micromethod of Brink et al. (1954). The low plasma levels of ethanol forced the introduction of modifications in the method consisting the reduction of the assay volume to 2 mL, whereas keeping the plasma sample in 100 μL. With these modifications, the lowest detected ethanol levels were below 0.1 mM or 0.59 μg/100 μL.

Basic hematology, blood biochemistry, and blood lipids determination

Complete EDTA–blood was used for cellular blood counting in an Advia® Flow Cytometer (Bayer AG, Leverkusen, Germany), whereas heparinized plasma was used to determine glucose, hepatic enzymes, and lipids in a modular turbidimeter analyzer (Roche Diagnostics, F. Hoffmann-La Roche Ltd., Basel, Switzerland).

Determination of plasma nitrites and nitrates

Plasma was obtained as described previously and was assayed immediately or stored at −80°C. A method (Granger et al., 1996) based in Griess reaction was used to measure nitrites and nitrates.

Determination of endothelial nitric oxide synthase in the lung

The endothelial nitric oxide synthase (eNOS) protein present in lung homogenates was determined by Western blot. Lung protein samples (50 μg of protein determined by Lowry) were separated by *sodium* dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (MiniProtan®, Bio-Rad) and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore Corporation, MA). PVDF membranes were probed with mouse monoclonal or rabbit antibodies (Becton, Dickinson and Company [BD]; New Jersey, NJ) against eNOS and inducible nitric oxide synthase (iNOS) (diluted at 1:400 or 1:500). As second antibodies, we used anti-mouse or anti-rabbit IgG-horseradish