Alcohol 61 (2017) 25–31

Contents lists available at ScienceDirect

Alcohol

journal homepage: http://www.alcoholjournal.org/

Effects of moderate alcohol consumption on gene expression related to colonic inflammation and antioxidant enzymes in rats

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ARTICLE INFO

Article history:
Received 11 August 2016
Received in revised form
11 February 2017
Accepted 14 February 2017

Keywords:
Colorectal cancer
Alcohol
Moderate
Inflammation
DNA damage

ABSTRACT

Excessive alcohol consumption is a risk factor associated with colorectal cancer; however, some studies have reported that moderate alcohol consumption may not contribute additional risk for developing colorectal cancer while others suggest that moderate alcohol consumption provides a protective effect that reduces colorectal cancer risk. The purpose of this study was to determine the effects of moderate voluntary alcohol (20% ethanol) intake on alternate days for 3 months in outbred Wistar rats on risk factors associated with colorectal cancer development. Colonic gene expression of cyclooxygenase-2, RelA, 8-oxoguanine DNA glycosylase 1, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase M1, and aldehyde dehydrogenase 2 were determined. Blood alcohol content, liver function enzyme activities, and 8-oxo-deoxyguanosine DNA adducts were also assessed. Alcohol-treated rats were found to have significantly lower 8-oxo-deoxyguanosine levels in blood, a marker of DNA damage. Alanine aminotransferase and lactate dehydrogenase were both significantly lower in the alcohol group. Moderate alcohol significantly decreased cyclooxygenase-2 gene expression, an inflammatory marker associated with colorectal cancer risk. The alcohol group had significantly increased glutathione-S-transferase M1 expression, an antioxidant enzyme that helps detoxify carcinogens, such as acetaldehyde, and significantly increased aldehyde dehydrogenase 2 expression, which allows for greater acetaldehyde clearance. Increased expression of glutathione-S-transferase M1 and aldehyde dehydrogenase 2 likely contributed to reduce mucosal damage that is caused by acetaldehyde accumulation. These results indicate that moderate alcohol may reduce the risk for colorectal cancer development, which was evidenced by reduced inflammation activity and lower DNA damage after alcohol exposure.

Introduction

Colorectal cancer (CRC) is ranked third in the leading cancer types for estimated new cases and deaths for both genders in the United States (Siegel, Miller, & Jemal, 2015). The American Cancer Society estimates 69,090 and 63,610 new cases and 26,100 and 23,600 deaths in 2015 for men and women, respectively (Siegel et al., 2015). For most individuals, the development of CRC is sporadic, and risk factors include consuming a high-fat diet, low fiber intake, sedentary lifestyle, obesity, diabetes mellitus, smoking, and excessive alcohol intake (Cunningham et al., 2010).

Prior epidemiological studies have investigated the dose-response relationship between alcohol consumption and CRC risk. Recently, a meta-analysis found that alcohol consumption was positively associated with CRC risk with estimated relative risks of 1.03 (95% confidence interval [CI] 0.92–1.20) for 10 g/day, 1.08 (95% CI 1.02–1.19) for 25 g/day, 1.14 (95% CI 1.07–1.21) for 50 g/day, and 1.43 (95% CI 1.25–1.64) for 100 g/day alcohol consumption (Wang, Duan, Yang, & Lin, 2015). However, the authors acknowledged that the observed dose-response relationship could be prone to confounding due to the meta-regression analysis used, and that the results were exploratory in nature (Wang et al., 2015). Conversely, a recent review concluded that increased risk was significant only for individuals consuming above a threshold of 30 g of alcohol per day, which is equivalent to two standard alcoholic beverages (Klarich, Brasser, & Hong, 2015). Another study found significant reduced

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http://dx.doi.org/10.1016/j.alcohol.2017.02.179
0741-8329/

risk of CRC in a Mediterranean population that consumed moderate amounts of alcohol, between 12 and 35 g of alcohol per day (Kontou et al., 2012). However, other dietary factors may have influenced these results, indicating that moderate alcohol consumption provided a protective effect that reduced CRC development.

Uncertainty remains regarding the role of moderate alcohol consumption on risk for CRC. Highly controlled investigations that aim to determine risk factors that may influence the development of cancer are difficult to conduct in human subjects (Lu et al., 2014). Animal studies are critical to better understand the underlying mechanisms mediating effects of a given substance on cancer risk, as the animal studies allow for controlled examination of normal physiological states that may become altered from increased inflammation and DNA damage that precede cancer development (Lu et al., 2014).

Numerous preclinical studies have been used to investigate the relationship between alcohol consumption and CRC risk. Various approaches have been used in animal models, including administration of a pro-carcinogenic agent to attempt to induce CRC development, as well as the use of genetically modified strains of mice that are prone to developing intestinal tumors (Hamilton, Sohn, & Fiala, 1987; Hayashi et al., 2007; Kushida et al., 2009; Perse & Cerar, 2007; Pérez-Holanda, Rodrigo, Viñas-Salas, & Piñol-Felis, 2005; Roy et al., 2002; Wimberly et al., 2013). Results from these studies vary considerably and many limitations have been reported with their use (Perse & Cerar, 2007). For instance, Perse and Cerar (2007) advise against the use of 1,2-dimethylhydrazine and azoxymethane for animal models of CRC when administering alcohol, due to variation among studies in the timing of carcinogen administration and the dose of alcohol administered. Additionally, many studies have administered an excessive dose of alcohol to reflect the effect of chronic alcohol consumption in humans (Choi et al., 1999; Simanowski et al., 1994; Wimberly et al., 2013). However, the current literature assessing the effects of moderate alcohol on CRC development in rodent models is limited. Additional controlled preclinical studies are needed that investigate the influence of moderate alcohol exposure on physiologic changes related to CRC development and to characterize biomarkers that may be mechanistically involved in disease risk.

The aim of the current study was to investigate the effects of moderate voluntary alcohol consumption in outbred Wistar rats on several risk factors associated with CRC development. These included examining interactions between alcohol and cyclooxygenase-2 (COX-2), RelA, CD68, myeloperoxidase (MPO), the repair of 8-oxo-deoxyguanosine (8-oxo-dG) by 8-oxoguanine DNA glycosylase 1 (OGG-1), the antioxidant activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase M1 (GSTM1), and expression of aldehyde dehydrogenase 2 (ALDH2). Additionally, liver function enzyme tests including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK), and gamma-glutamyltransferase (γ-GT) were measured under non-intoxicated conditions (i.e., nonsignificant blood alcohol content). The goal was to provide a more thorough understanding of the effects of moderate alcohol consumption on measures related to CRC development and to identify potential mechanisms mediating disease risk.

Materials and methods

Animals and diets

This study was approved by the Institutional Animal Care and Use Committee at San Diego State University and was in accordance with National Institutes of Health guidelines. Naïve adult male outbred Wistar rats ($n = 24$; 503.0 ± 10.9 g, Harlan Laboratories, Placentia, CA, USA) at 109 days of age were randomly assigned to two groups ($n = 12$/group). The experimental group was fed a regular chow diet (LabDiet, St. Louis, MO, USA), was given water *ad libitum*, and was provided access to a 20% ethanol solution (Pharmco-Aaper, Brookfield, CT, USA) on alternate days for 13 weeks (details below; Simms et al., 2008). In the control diet, rats were fed the same chow and drank exclusively water. Both chow diets contained 28.5% of energy as protein, 13.5% of energy as fat, and 58% of energy as carbohydrates. All rats were fed *ad libitum* throughout the experiment. During the course of experimental procedures, animals were housed individually in standard tub cages in a vivarium that maintained a 12:12-h light/dark cycle and an ambient temperature of approximately 23 °C.

Ethanol exposure

Rats were exposed for 13 weeks to either a 20% ethanol intermittent-access drinking paradigm ($n = 12$) or were given access only to water ($n = 12$). This alcohol exposure paradigm has previously been shown to result in blood alcohol concentrations (BACs) averaging ~30–50 mg/dL in outbred Wistar rats when measured 30–120 min into a standard drinking session (Cippitelli et al., 2012; George et al., 2012; Simms et al., 2008). The first seven daily sessions served to acclimate the animals to the testing procedures and all rats received free access to food and water only. Following the acclimation period, ethanol-exposed rats began 22-h intake sessions involving voluntary access to a 20% (v/v) ethanol solution vs. water, alternating with 22-h abstinence periods involving voluntary access to water only (45 ethanol-drinking sessions total, over 13 weeks). The positions of ethanol and water bottles were rotated each ethanol session to control for position preferences. The control group was given voluntary access to water only during the entire duration of the chronic exposure period. All fluids were weighed to the nearest gram and replaced daily, and body weights were measured every 48 h. One day following their final experimental session, rats were sacrificed via carbon dioxide asphyxiation and tissues were collected for subsequent analysis.

Sample collection

Blood was collected after euthanasia and was centrifuged at 1200×*g* for 15 min at 4 °C; serum was stored at −80 °C. The colorectum was excised, opened longitudinally, and the feces pushed off gently. It was then washed with phosphate buffered saline (PBS) and the mucosa was scraped off gently with glass microscope slides. Colonic mucosal scrapings were frozen and stored at −80 °C.

Blood alcohol content

Blood alcohol content following sacrifice was assessed in serum using the Ethanol Assay Kit (Sigma-Aldrich, St. Louis, MO, USA). The protocol indirectly determined ethanol concentration by a coupled enzyme reaction, which results in a colorimetric (570 nm) product, proportional to the ethanol present.

Liver enzyme tests

Liver enzyme concentrations, including ALT and AST, were measured in serum using ALT/GPT (UV-Rate) (Stanbio, Boerne, TX, USA) and AST/GOT (UV-Rate) (Stanbio, Boerne, TX, USA), respectively. The Alkaline Phosphatase LiquiColor® Test (Stanbio, Boerne, TX, USA) was used to measure ALP, and γ-GT LiquiColor® Test (Stanbio, Boerne, TX, USA) was used to measure γ-GT in the serum. Lactate