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The effects of ethanol on angiogenesis after myocardial infarction, and preservation of angiogenesis with rosuvastatin after heavy drinking

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ABSTRACT

The cardioprotective effects of moderate alcohol consumption and statins have been known for years. However, heavy or binge drinking confers a high risk of cardiovascular disease. This study aimed to investigate the effects of different levels of alcohol consumption on acute myocardial infarction that was induced experimentally in rats, with a focus on the potential mechanism of angiogenesis and the effects of statins on heavy drinking. The experimental rats were fed low-dose ethanol (0.5 g/kg/day), high-dose ethanol (5 g/kg/day), and high-dose ethanol with rosuvastatin (10 mg/kg/day) during the entire experiment. Acute myocardial infarctions were induced 4 weeks after the beginning of the experiment. We assessed the capillary density in the myocardium via immunohistochemistry and quantified the expression of vascular endothelial growth factor (VEGF) and endostatin via enzyme-linked immunosorbent assay kits on the 4th day after myocardial infarction. The results revealed that low ethanol consumption promoted angiogenesis in association with higher VEGF and lower endostatin. High ethanol intake suppressed angiogenesis with unchanged VEGF and elevated endostatin. Treatment with rosuvastatin preserved angiogenesis following high ethanol intake, with an upregulation of VEGF. This study highlights that low ethanol consumption obviously promotes angiogenesis in myocardial-infarction rats while increasing the expression of VEGF, whereas high ethanol consumption inhibits ischemia-induced angiogenesis. This study also provides evidence that rosuvastatin alleviates the inhibitory effects of heavy drinking on angiogenesis.

Introduction

Cardiovascular disease is the leading cause of death in industrialized countries. Myocardial infarction is the common presentation of cardiovascular diseases and has a high mortality rate (Mozaffarian et al., 2015). Angiogenesis is defined as the formation of new blood vessels from pre-existing vessels. Angiogenesis plays a protective role in many ischemic diseases, including myocardial infarction and associated remodeling (Iribarren et al., 2011). Ischemia-induced angiogenesis is a compensatory response that re-establishes the blood supply to ischemic tissues. The promotion of angiogenesis has been accepted as a promising therapy for ischemic diseases (Freedman & Isner, 2001). In ischemic tissues, angiogenesis mainly depends on the expression of some important angiogenic factors, such as VEGF and endostatin. Many studies have reported that endostatins are negatively correlated with coronary collaterals in chronic myocardial ischemia and remarkably increase following acute myocardial infarction (Seko, Fukuda, & Nagai, 2004).

Many epidemiological studies have demonstrated that the relation between alcohol consumption and the risk of coronary heart disease is J-shaped (Ruidavets et al., 2010). Moderate ethanol consumption is associated with a low risk of coronary heart disease, whereas heavy or binge drinking confers a high risk of cardiovascular events (Razvodovsky, 2013). Generally, the beneficial effects of low-dose ethanol consumption are ascribed to increases in the levels of high-density lipoprotein cholesterol with adiponectin, and reductions in the levels of fibrinogen (Brien, Ronksley, Turner, Mukamal, & Ghali, 2011). Another study revealed that moderate alcohol intake results in changes in inflammatory mediators, such as C-reactive protein (Zairis et al., 2004). The mechanisms by which alcohol intake influences cardiovascular disease are not yet well known. Based on evidence that angiogenesis plays a protective role

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in myocardial infarction (Fujita & Sasayama, 2010), the present study was designed to examine the effects of different doses of ethanol consumption on angiogenesis in acute myocardial infarction in rats.

We hypothesized that low-dose and high-dose alcohol consumption would have opposite effects on angiogenesis because of the dose-response relationship between alcohol consumption and cardiovascular diseases. Clinical trials have demonstrated the benefits of statins on coronary heart disease and strokes, due to reductions in cholesterol levels (Collins et al., 2003). Statins have been demonstrated to exhibit other cardioprotective roles that are unrelated to their lipid-lowering effects. These protective roles include the inhibition of myocardial fibrosis (Mannheim et al., 2011) and the induction of angiogenesis following myocardial infarction (Zaitone & Abo-Gresha, 2012). In this study, we intended to investigate whether rosuvastatin alleviated the undesirable effects of heavy drinking on angiogenesis in rats with myocardial infarction.

Methods

Drugs and reagents

Rosuvastatin was purchased from AstraZeneca (London, UK). Fifty percent ethanol and CD34 antibody were obtained from Boster (Wuhan, China). Horseradish peroxidase-conjugated secondary antibodies were purchased from ZSGB-BIO (Beijing, China). The endostatin ELISA and VEGF ELISA kits were obtained from Bio-Swamp (Shanghai, China) and USCN Life Science (Houston, Texas, USA), respectively.

Animals and groups

Male adult Sprague–Dawley rats (225–275 g) were used in the present study. All procedures were approved by the Animal Care Committee of Shandong University and conformed to the Guide for the Care and Use of Laboratory Animals (1996). Forty rats were randomly divided into five groups of eight rats each. The sham-operation control group and water-control group rats received normal diets. The low-dose ethanol group and high-dose ethanol group rats received ethanol (0.5 g/kg/day or 5 g/kg/day) once per day. The rosuvastatin + high-dose ethanol group rats received ethanol (5 g/kg/day) and rosuvastatin (10 mg/kg/day) once per day. In the present study, all ethanol and rosuvastatin doses were administered by gastric gavage.

Animal model of myocardial infarction

After 4 weeks, all rats were anesthetized with 3% chloral hydrate administered via intraperitoneal injection. After a rodent ventilator (ALCBIO, Shanghai, China) was applied for tracheal intubation, and electrocardiographic (ECG) monitoring was achieved, the heart was fully exposed. At the midpoint of the anterior descending left coronary artery, ligation was achieved with a 6-0 silk suture. ST segment elevation and pathological Q waves in the electrocardiographs (ECG) were monitored. For the sham group, a suture was placed under the left anterior descending aspect of the coronary artery without ligation. Next, the chests were closed, and the animals were allowed to recover in a small-animal intensive care unit. All the rats after surgery were fed as before.

Specimen collection

On the fourth day after myocardial infarction, the rats were anesthetized via intraperitoneal injection of 3% chloral hydrate (0.35 mL/100 g). The heart was removed, trimmed of the connecting tissue, and rinsed with ice-cold phosphate-buffered saline (PBS). The left ventricle was weighed to calculate the ratio of the left ventricular weight to the body weight. This ratio was determined as an index of cardiac hypertrophy. The ventricular myocardium was divided into two parts. One part was maintained at −80 °C for the measurement of the endostatin and VEGF levels. The other part was fixed with 4% paraformaldehyde for 48 h and then embedded in paraffin. All myocardial tissues were sectioned and dried overnight at 37 °C. Next, the sections were washed in PBS and incubated in 1% bovine serum albumin (BSA) for 30 min. Finally, the sections were stored for immunohistochemical CD34 assessment.

Immunohistochemistry

As mentioned above, after deparaffinization and rehydration, the myocardial tissue sections were incubated overnight at 4 °C with the rat primary antibody against CD34 (1:100) plus 1% BSA in PBS. The control sections were incubated in PBS alone. Next, the sections were incubated in biotinylated goat anti-rat secondary antibody (1:200 in PBS) and subsequently incubated in an avidin-horseradish peroxidase solution. The immunostaining was visualized with 0.05%diaminobenzidine plus 0.3% H_2O_2 in PBS. The sections were examined with a light microscope (Olympus, Japan).

The numbers of CD34-positive microvessels were measured in each section using the well-known "hot spot" method described by Weidner, Semple, Welch, and Folkman (1991). After the areas of highest neovascularization were found by scanning the section at low power (100×), individual microvessel counts were made on a 200× field. Vessels were counted in five high-power fields (HPF 200×). An average of multiple fields was calculated to represent the microvessel density (MVD). Any highlighted endothelial cell or endothelial cell cluster clearly separate from adjacent microvessels, myocardial cells, and other connective tissue elements was considered a single, countable microvessel. All the histological examinations were performed blindly.

Determinations of endostatin and VEGF

Each frozen cardiac sample was homogenized in PBS solution (pH 7.4) using a homogenizer and then centrifuged at 1500 rpm for 15 min at 4 °C to remove the debris. The supernatants were collected and divided into portions. Next, the tissue levels of endostatin and VEGF in the myocardia were determined using immunodiagnostic kits for endostatin and VEGF, obtained from Bio-Swamp and USCN Life Science, respectively. All of the examinations were performed following the manufacturers' instructions.

Statistical analysis

SPSS version 18.0 (SPSS, Inc., Chicago, IL, USA) was used for the statistical analysis. All of the data were recorded and are expressed as the mean ± S.E.M. Comparisons among multiple groups were performed with one-way repeated-measures analysis of variance (ANOVA), and pairwise comparisons were performed with LSD-q tests. p values < 0.05 were considered statistically significant.

Results

Effect of different doses of ethanol and rosuvastatin on the survivals of the experimental animals

In the present study, myocardial-infarcted rats were created by ligating the anterior descending coronary artery. By the end of the