Renal Oxidative Stress in Chronic Alcohol-Induced Hypertension

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Chronic high-dose alcohol consumption increases mortality and causes cardio-renal diseases including hypertension. Oxidative stress to the kidney may play an important role in the regulation of blood pressure. Our project tested the hypothesis that chronic alcohol ingestion in rats exerts oxidative stress in the renal system, which in turn causes activation of the renin-angiotensin system leading to an increase in blood pressure.

Male Fisher rats were fed daily ethanol at a dose of 4 g/kg for 12 weeks and controls received 5% sucrose daily for 12 weeks. The blood pressure was recorded weekly through the tail-cuff method. At the end of the treatment, the rats' kidneys were isolated and analyzed for oxidative stress parameters. Chronic alcohol administration caused a profound increase in mean blood pressure (BP) 140±8 mm Hg compared to the control levels of 100±9 mm Hg. The increase in BP was correlated with increased malondialdehyde (MDA) levels (124% of control), depression of the reduced-to-oxidized-glutathione ratio (GSH/GSSG) and antioxidant enzyme activities of CuZn-SOD (73% of control), catalase (71% of control) and glutathione peroxidase (34% of control) in the kidneys of alcohol-treated rats. The renal Mn-SOD activity was significantly increased (150% of control) in chronic alcohol-treated rats.

The study concluded that chronic, high dose ethanol caused hypertension, which was related to oxidative stress (depletion of antioxidant enzyme activities, GSH/GSSG ratio and enhanced lipid peroxidation) to the kidneys of rats.

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Introduction

The molecular mechanisms and endogenous mediators responsible for alcohol-induced hypertension and associated renal injuries are not clear. The kidneys contribute to the maintenance of blood pressure by regulating the volume of intravascular fluid through the renin-angiotensin-aldosterone system.[1] Chronic alcohol ingestion can cause oxidative injury to the renal system through enhanced free radical/reactive oxygen species (ROS) generation and depletion of antioxidant defense system. These reactive species oxidize cellular bio-molecules and initiate membrane lipid peroxidation leading to cellular dysfunction. However, the cell is endowed with an elaborate antioxidant defense system to protect against free radical damage. These include antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and the most important endogenous antioxidant, glutathione (GSH). The oxidant/antioxidant balance in the renal system has an important role in protecting the renal tissues, thus allowing normal blood pressure. However, mechanisms underlying the renal injury and redox regulation of alcohol-induced hypertension have not been fully explored, but are the subject of this investigation.

Methods

Male Fisher rats (body weights of 200–250 g) were used in the study. The first group of rats ($n = 6$) were treated with 5% sucrose (orally) daily for 12 weeks and served as the control group while the second group of rats ($n = 6$) were fed alcohol orally at a dose of 4g/kg daily for 12 weeks. The systolic, diastolic and mean blood pressures were

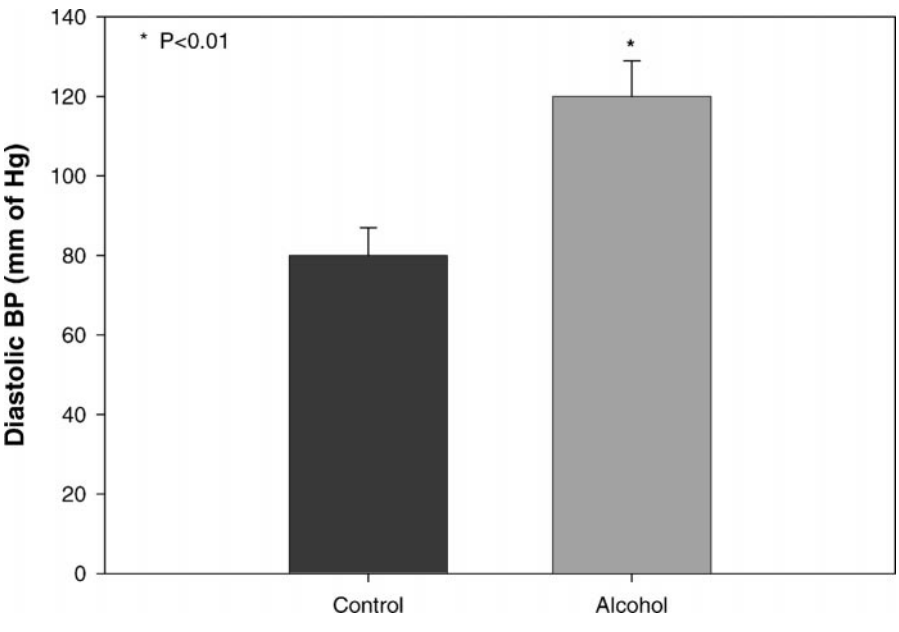

Fig 1. Diastolic blood pressure in rats

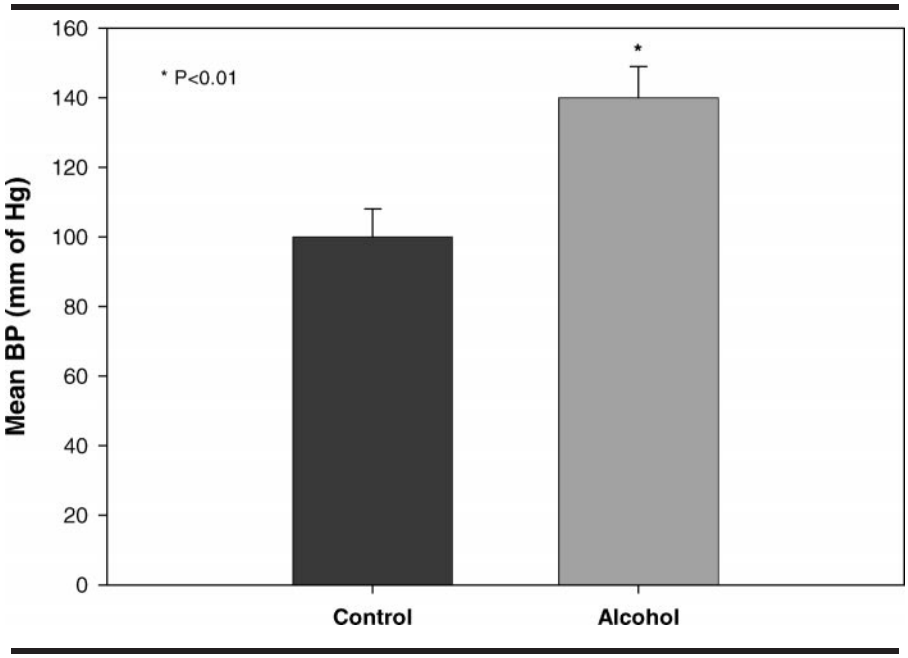

Fig 2. Mean blood pressure in rats

recorded every week through tail-cuff method using NIBP-8 monitor (Columbus Instruments, OH). After treatment, the kidneys were isolated and analyzed.

The tissues were homogenized in cold 50 mM phosphate buffer pH 7.0 and centrifuged at 3000 rpm for 10 min. The supernatant was used for biochemical assays. The antioxidant enzymes such as cytosolic CuZn-SOD and mitochondrial Mn-SOD activities were determined by the method of Misra and Fridovich,[2] catalase (CAT) activity by the method of Flohe and Gunzler,[4] and NADPH oxidase activity as described by Cui and Douglas.[5] The end product of lipid peroxidation malondialdehyde (MDA) was determined by the method of Ohkawa et al[6] and protein concentration by the method of Read and Northcole.[7] The reduced-to-oxidized-glutathione ratio (GSH/GSSG) was analyzed using commercial kit from The Trevigene, MD, USA.

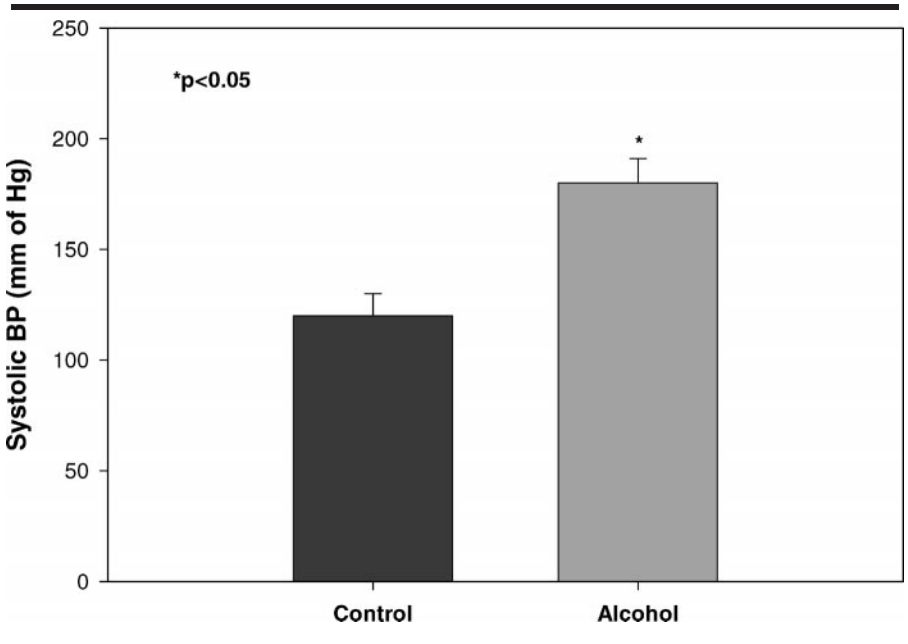

Fig 3. Systolic blood pressure in rats

Data Analysis

The data are expressed as mean + SEM and analyzed statistically using Student's *t* test. The .05 level of probability was used as statistical significance.

RESULTS

Systolic, diastolic and mean blood pressure were significantly increased in the alcohol-fed group compared to control group (Figure 1, 2 and 3).

The lipid peroxidation end product (MDA) significantly increased in alcohol-fed group compared to control group. The NADPH oxidase activity significantly increased in the alcohol-fed group compared to control group. Reduced-to-oxidized-glutathione ratio significantly decreased in the alcohol-fed group compared to control group.

Table 1 demonstrates the effect of alcohol (4g/kg) oral administration for 12 weeks on antioxidant enzymes activity in the kidney of rats. Activities such as Cu-Zn SOD, CAT and G-Px were significantly decreased in the alcohol-fed group compared to the control group, while Mn-SOD activity significantly increased in the alcohol-fed group compared to control group.

CONCLUSION

The study concluded that chronic, high-dose ethanol ingestion caused hypertension, which was related to oxidative stress (depletion of antioxidant en-

Table 1. The effect of alcohol on antioxidant enzymes activity in the kidneys of rats

Antioxidant Enzymes	Control ($n = 6$) (mean ± SE)	Alcohol ($n = 6$) (mean ± SE)
Cu Zn-SOD	71.55 ± 4.91	52.59 ± 4.56*
Mn-SOD	50.44 ± 4.64	75.32 ± 4.69†
CAT	60.81 ± 4.00	43.12 ± 3.27‡
G Px	23.69 ± 3.75	8.09 ± 2.65§

* Significant as compared to control ($P<.05$).
† Significant as compared to control ($P<.02$).
‡ Significant as compared to control ($P<.01$).
§ Significant as compared to control ($P<.001$).
Cu Zn-SOD = Copper Zinc Superoxide Dismutase. Mn-SOD = Manganese Superoxide Dismutase, CAT = Catalase. G Px = Gluthathione Peroxidase. SOD = Units/mg protein, CAT = mM H_2O_2 degraded/min/mg protein. G Px = μ moles of NADPH oxidized/min/mg protein.

zyme activities, GSH/GSSG ratio and enhanced lipid peroxidation) to the kidneys of rats.

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