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Recommended Citation
Miyamae, Masami; Kaneda, Kazuhiro; Domae, Naochika; and Figueredo, M.D., Vincent M., "Cardioprotection by regular ethanol consumption: potential mechanisms and clinical application." (2010). *Cardiology Faculty Papers.* Paper 15.
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**As submitted to:**

**Current Drug Abuse Reviews**

**And later published as:**

# Cardioprotection by Regular Ethanol Consumption: Potential Mechanisms and Clinical Application

**Volume 3, Issue 1, 2010, Pages 39-48**

**PubMed ID: 20230376**

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Conflict of Interest: The authors have no conflicts of interest to report.

Key Words: ethanol, ischemia-reperfusion injury, preconditioning, heart

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Running Title: ethanol in cardiac ischemia-reperfusion injury

# Abstract

Epidemiological studies demonstrate that excessive drinking is associated with hypertension, cerebral bleeding and loss of cardiac contractility. Conversely, studies have shown that mortality rates for people who regularly drink ethanol in moderation are lower than in abstainers, primarily due to decreased fatal ischemic heart disease. Further, moderate ethanol consumers have lower rates of myocardial infarction compared with abstainers. These beneficial cardiac effects may be due to pleiotropic effects of ethanol on lipids, platelets, and fibrinolytic activity. During the past decade, studies conducted in several animal models have revealed that light to moderate regular ethanol consumption renders hearts more tolerant to myocardial ischemia-reperfusion injury; to a degree similar to cardiac ischemic preconditioning (brief episodes of ischemia dramatically limit infarct size following prolonged ischemia). Recent clinical evidence suggests that light to moderate ethanol consumption in the year prior to myocardial infarction is associated with reduced mortality following myocardial infarction. These findings suggest that light to moderate ethanol consumption not only prevents myocardial infarction but also improves survival after myocardial infarction. Proposed mechanisms of cardioprotection by regular ethanol consumption include activation of adenosine A1 receptors, $\alpha_1$-adrenoceptors, protein kinase C-δ and ε, adenosine triphosphate-dependent potassium ($K_{ATP}$) channels, nitric oxide synthase and reduced leukocyte-endothelial cell adhesive interactions. In this review, we focus on the recent progress in elucidating the endogenous myocyte signaling mediating cardioprotection by light to moderate ethanol consumption.

# INTRODUCTION

The link between ethanol and cardiovascular disease is important because diseases affecting the circulatory system are a leading cause of mortality in many developing countries. Numerous studies have demonstrated that excessive drinking is associated with cardiovascular disorders, including cardiomyopathy, hypertension, arrhythmia, coronary heart disease, cerebral bleeding and other life-threatening medical complications [1-3]. Especially, ischemic heart disease is most important all over the world. Since ancient times, however, it has long been recognized that ethanol in moderation is healthy; ethanol abuse detrimental. In the past century, epidemiological studies have shown that mortality rates for people who regularly drink ethanol in moderation are lower than in abstainers [1,2,4,5]. This is primarily due to decreased incidence of fatal ischemic heart disease [6,7]. The beneficial cardiac effects of moderate ethanol consumption may be due to the pleiotropic effects of ethanol on lipids [8], platelets, and fibrinolytic activity [9,10]. Even very low consumption of ethanol (one or two drinks per week) has been shown to be cardioprotective [11].

Recent clinical studies demonstrated that light to moderate ethanol consumption may provide further cardioprotection, attenuating ischemia-reperfusion injury and improving outcome after acute myocardial infarction [12-16]. Moderate ethanol consumers improved survival after myocardial infarction compared with abstainers [13]. During the past decade, studies conducted in several animal models have revealed that light to moderate regular ethanol consumption renders hearts more tolerant to myocardial ischemia-reperfusion injury to a degree similar to cardiac ischemic preconditioning [17,18], in which brief episodes of ischemia and reperfusion dramatically limit infarct size following prolonged ischemia [19].

We have termed this protective effect of ethanol against ischemia-reperfusion injury “ethanol preconditioning” [17].

Recognizing the deleterious effects of ethanol abuse on the heart and other organ systems, in this review, we discuss two potential beneficial effects of light to moderate ethanol consumption on the heart; prevention of coronary heart disease and reduction of ischemia-reperfusion injury. We review pleiotropic effects of ethanol which may contribute to decreased development of ischemic heart disease. We then discuss the endogenous myocyte signaling likely mediating ethanol cardioprotection against ischemia-reperfusion injury.

## Pleiotropic Effects of Ethanol: prevention of ischemic heart disease

Clinical and epidemiologic studies show that moderate drinkers have less cardiovascular disease when compared with nondrinkers or heavy drinkers [1-3,20,21]. This is primarily due to a decreased incidence of coronary heart disease which results in a U-shaped or J-shaped curve between ethanol consumption and mortality. Plausible mechanisms for this cardioprotection, as well as adverse effects of ethanol abuse, are shown in Figure 1. These mechanisms include increases in high density lipoprotein cholesterol (HDL-C), decreases in low density lipoprotein cholesterol (LDL-C) [22], decreased platelet aggregation, increased fibrinolytic activity, suppression of inflammatory cytokines and increased insulin sensitivity through increased levels of plasma adiponectin [23]. Ethanol consumption increases apolipoprotein A-I and A-II which are the main protein components of HDL-C [24]. Multiple

epidemiological studies have shown an inverse relationship between serum HDL-C (especially HDL3) level and morbidity due to ischemic heart disease [8,20,24-26]. Rimm at al., calculated that consumption of 30 g ethanol per day, regardless of beverage type, reduces ischemic heart disease events 16.8 % by increasing HDL-C [20]. Reports suggest that at least half of the beneficial effect of ethanol on atherosclerosis is attributed to increased HDL [27,28].

Rao et al. demonstrated that light, but not heavy, ethanol consumption upregulates paraoxonase 1 expression [32]. Increased serum paraoxonase has been shown to limit LDL-C peroxidation and play a major role in the protective effect of HDL against coronary artery disease [33,34]. Ethanol induces qualitative changes on HDL-C which results in phospholipids enrichment. This is thought to reduce the inflammatory response of atherogenesis [35]. Anti-inflammatory effects by moderate ethanol consumption are well established. Imhof et al., studied a random cross-sectional sample of over 8000 men and women in three European countries to investigate the effect of ethanol consumption on inflammatory markers including white blood cell counts, fibrinogen and C-reactive protein (CRP). Moderate ethanol consumers was associated with lower levels of these markers [36].

Moderate ethanol consumption may have beneficial effects on the vascular system, reducing CRP and adhesion molecules, including intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM) and E-secletin [37,38]. Pai et al., studied over

1400 healthy men and women from a large prospective study. They found significant trends between increasing ethanol intake and soluble tumor necrosis factor-α (TNF-α) receptor 1 and 2 . Compared with abstainers, men who consumed on average 1-2 drinks/day had 26% lower CRP and 36% lower interleukin-6, a proinflammatory cytokine. A similar association was observed in women who consumed half drink per day [39].

Other proposed mechanisms for the protective effect of moderate ethanol consumption against ischemic heart disease events include beneficial effects on hemostasis [40], endothelial function and insulin resistance [41]. Moderate ethanol consumption has been shown to affect several hemostatic factors, including fibrinogen concentration, platelet aggregability, tissue plasminogen activator (t-PA) and plasminogen activator inhibitor [20,42-44]. Moderate ethanol consumption decreases platelet aggregation, increases fibrinolytic activity and reduces fibrinogen levels, as well as levels of the Von Willebrand factor, important in platelet adhesion and aggregation [45]. Endothelial cells also have been reported to play an important role in increased fibrinolytic activity induced by ethanol consumption [46]. Booyse et al., demonstrated that fibrinolytic protein such as t-PA expression is modulated by ethanol and wine polyphenols. Clot lytic rates significantly increased in mice treated with either ethanol, catechins and quercetin [30]. Nitric oxide (NO) produced from endothelial cell inhibits smooth muscle cell proliferation and platelet aggregation [47].

Furuya et al. demonstrated that there is an inverted U-shaped relationship between ethanol intake and insulin sensitivity in an experimental model [49]. Bell et al., assessed the relationships between ethanol consumption and insulin sensitivity and coronary risk factors in a cross-sectional analysis of 1,196 subjects. They found that moderate ethanol

consumption was associated with improved insulin sensitivity and modifiable cardiac risk factors such as lipids and blood pressure [41].

When considering the relationship between ethanol consumption and coronary heart disease, the role of confounding variables such as diet, physical activity and genetics cannot be ignored. Light to moderate ethanol consumption is associated with a lower prevalence of metabolic syndrome, with beneficial effects on lipids, waist circumference and fasting insulin [50]. Studies suggest that wine consumption is associated with a diet high in fruits, vegetables and fish and low in saturated fat (Mediterranean diet) [51,52] [53]. It has been demonstrated that the mortality of fatal ischemic disease is lower among the physically active who consume moderate ethanol [54].

Genetic differences may also contribute to the variability in metabolic capacity of ethanol among individuals. This may in turn influence the magnitude of cardioprotection afforded by moderate ethanol consumption. Ethanol is metabolized by alcohol dehydrogenase (ADH) and the mitochondrial form of aldehyde dehydrogenase (ADH2) [55,56] The polymorphism of encoded genes for these enzymes changes their activity and the frequencies of these variants highly depend on the ethnicity.

Finally, non-alcoholic components such as wine polyphenols and dietary isohumulones (the bitter components of beer) have been implicated in the beneficial effects of alcohol beverage on atherosclerosis [30,31]. However, it appears that their contribution to cardioprotection is minor comparedto the effects of ethanol.

## Ethanol Preconditioning: attenuating cardiac ischemia-reperfusion injury

We discovered that regular ethanol consumption renders hearts more tolerant to ischemia-reperfusion injury mimicking cardiac ischemic preconditioning. This protection against ischemia-reperfusion injury is mediated through adenosine A1 receptor activation [17]. In these early studies, guinea pigs were treated with 2.5-20% ethanol in their drinking water for 3-12 weeks. This corresponds to a range equivalent to mild to heavy ethanol consumption. Isolated hearts were perfused and subjected to 45 min of no-flow ischemia and 48 min reperfusion. The effect of varying concentrations of ethanol and duration of exposure is shown in Table 1. Treatment for 3 weeks with 10% ethanol in their drinking water induced cardioprotection. As little as 2.5% ethanol in the drinking water for 3 weeks protects only against a rise in left ventricular end-diastolic pressure (LVEDP) during reperfusion. However, by 6 weeks of treatment, full protection as indicated by improved left ventricular developed pressure (LVDP) recovery, decrease in LVEDP and creatine kinase release was seen regardless of dose of ethanol. The magnitude of this protection is similar to that of ischemic preconditioning elicited by 2 min of global ischemia and 5 min of reperfusion immediately before sustained ischemia in the absence of ethanol (Table 1). These salutary effects of ethanol consumption were abolished by the selective adenosine A1 receptor inhibitor, 8-cyclopentyl-1,3,dipropylxanthine (DPCPX), but not the A2 receptor inhibitor, 3,7-dimethyl-1-propargylxanthine (DNPX). This suggests that the adenosine A1 receptor, but not A2 receptor, plays a crucial role in cardioprotection afforded by chronic ethanol consumption. In another study, we reported that the mechanism of a cardioprotection by regular ethanol consumption can vary by species. In rats, ethanol

preconditioning was mediated by $\alpha_1$-adrenergic signaling, not adenosine A1 receptor signaling[57]. We also demonstrated that chronic ethanol exposure induces sustained translocation of ε-protein kinase C (PKC) in myocytes from the cytosolic to particulate fractions, but not α-PKC and δ-PKC. Isolated myocytes from hearts exposed to ethanol showed immunolocalization of ε-PKC antibody fluorescence from the perinuclear and cytosolic regions to the cross-striations (possibly myofilaments), suggesting that ε-PKC had translocated to activation sites, binding to anchoring molecules (receptors for activated C kinase or RACKs). These data correlated with the data obtained by Western blot analysis [58]. Translocation of PKC has been to play a crucial role in triggering the signal transduction responsible for ischemic preconditioning [59]. Data from most other laboratories corroborate our data (Table 2). Guiraud et al. demonstrated that infarct size of the hearts from rat treated with 7% ethanol in their drinking water for 7 weeks was smaller than control after 30 min ischemia and 120 min reperfusion [60]. The combination of regular ethanol consumption and ischemia preconditioning further reduced infarct size. However, translocation of ε-PKC was not observed in rat myocytes. Pagel et al. employed the method of chronic, intermittent ethanol feeding to more fully mimic the clinical setting. Dogs were fed with ethanol (1.5g/day) mixed with dry food twice per day for 12 weeks. Infarct size of ethanol treated animals was significantly reduced compared with control [61]. Most, but not all, studies show that regular ethanol consumption protects myocardium against ischemia-reperfusion injury. Dow et al. failed to demonstrate reductions in myocardial infarct size in rats treated with ethanol (15% or 36% in their drinking water) for 16 weeks [62]. Gibson et al. also reported that moderate and heavy amounts of alcohol (defined as 20% and 35% of total caloric intake) do not improve, and even worsen functionary recover (increased diastolic stiffness) after 21.5 min of ischemia followed by 30 min reperfusion in isolated rat

hearts [63]. The discrepancy among these studies remains unclear, but might be due to differences in experimental models (Table 2).

## Potential Mechanisms of Ethanol Preconditioning

Numerous studies have been conducted to elucidate the mechanisms of preconditioning induced by regular ethanol consumption against ischemia-reperfusion injury in the past decade. The signal transduction by which regular ethanol consumption exerts a cardioprotective effect against ischemia-reperfusion injury is similar to that of ischemic preconditioning (Figure 2). Adenosine mediates many of the acute and long-term effects of ethanol on cellular and organ functions [64,65]. Ethanol has been shown to increase extracellular adenosine by inhibiting adenosine uptake, which results in activation of adenosine receptors [66]. Experiments in pigs [67], rabbits [68], dogs [69,70], and studies with human tissue [71] indicate that adenosine A1 receptors mediate ischemic preconditioning. Involvement of adenosine A1 receptor activation in cardioprotection by regular ethanol consumption has been demonstrated in guinea pigs [17] and dogs [61], but not in rats [57].

Chronic ethanol exposure in cells with adenosine A1 receptors causes hypersensitization of cAMP production [72], in contrast to cells expressing adenosine A2 receptors where ethanol causes heterologous desensitization of cAMP signal transduction [73,74]. In rats, $\alpha_1$-adrenergic receptors have been implicated in cardioprotection by regular ethanol consumption [57]. Stimulation of these G-protein coupled receptors by chronic ethanol exposure initiates production of the second messenger diacylglycerol, and results in

activation of phospholipase C [75] and PKC [58]. PKC signaling is crucial for triggering ethanol preconditioning [76]. Inagaki and Mochly-Rosen demonstrated that activation of δ-PKC induced by acute ethanol exposure mediated ε-PKC activation in ethanol-induced cardioprotection from ischemia [77]. Sustained translocation of ε-PKC from the cytosol to the myocyte membrane [58] or mitochondria [78] has been shown to play a role in ethanol preconditioning. ε-PKC primes both sarcolemmal [79] and mitochondrial [80] adenosine triphosphate-sensitive potassium channels (sarc$K_{ATP}$ and mito$K_{ATP}$) to open in ischemic preconditioning. Zhu et al. first demonstrated that mito$K_{ATP}$ channels are required for cardioprotection by chronic ethanol consumption in isolated rat hearts treated with 18% ethanol for 10 months [81]. Pagel et al. demonstrated that both sarc$K_{ATP}$ and mito$K_{ATP}$ play a role in reducing myocardial infarct size by chronic, intermittent ingestion of small amount of ethanol in vivo dog hearts [82].

NO is an important mediator in cardioprotection, activating mito$K_{ATP}$ [83]. Opening of mito$K_{ATP}$ increases levels of reactive oxygen species (ROS) in cardiomyocytes [84]. ROS activates a second pool of ε-PKC 1, designated ε-PKC 2, which inhibits the mitochondrial permeability transition. Mito$K_{ATP}$-generated ROS also activates ε-PKC 1 and induces phosphorylation-dependent mito$K_{ATP}$ opening. Thus mito$K_{ATP}$-dependent mito$K_{ATP}$ opening constitutes a positive feedback loop capable of maintaining the channel open after the stimulus is no longer present. This feedback pathway may be responsible for the lasting protective effect of preconditioning, known as the memory effect [85].

Ethanol has been shown to increase antioxidant enzymes such as superoxide dismutase and catalase in myocytes [86]. A recent *in vivo* mouse study revealed that activation of ALDH2 by acute ethanol exposure is involved in the detoxification of toxic

aldehyde such as 4-HNE which mediates oxidative damage through activated ε-PKC translocated to mitochondria [78].

NO increases ventricular function and serves as a signaling molecule at low concentrations [87,88], but triggers inflammation and suppresses contractile function at high concentrations [89]. Endothelial nitric oxide synthase (eNOS) is important for regulating basal vascular tone and blood pressure [90,91]. Cardiac myocytes also express eNOS [92] which plays a role in regulating myocardial contractile function and oxygen consumption [93] [94]. Knockout mice lacking eNOS suffer larger infarcts than wild type after ischemia [95]. eNOS overexpression in mice attenuates myocardial reperfusion injury [96]. Ethanol has been shown to increase NO production through modulation of eNOS expression [97]. Abou-Agag et al. reported that moderate ethanol consumption for 8 weeks (defined as 9% of total caloric intake) enhanced maximum vascular relaxation and increased plasma NO production concomitant with increase in eNOS protein in the vascular endothelium. In contrast, heavy ethanol consumption (defined as ~36% of total caloric intake) reduced maximum vascular relaxation [98]. We have recently demonstrated that ethanol preconditioning can be enhanced by administration of the volatile anesthetics, sevoflurane before ischemia through upregulation of eNOS by ethanol [99].

We have recently examined how long ethanol's cardioprotection persists after abstention and whether inducible NOS (iNOS) and/or eNOS play a role in this continued cardioprotection. Guinea pigs received 5% ethanol in their drinking water for 8 weeks. Isolated hearts were subjected to 30 min ischemia and 120 min reperfusion at 0, 4, 7 and 14 days after abstention. Contractile recovery after ischemia-reperfusion was significantly improved at 0, 4 and 7, but not 14 days of abstention, compared to controls. Western blot

analysis and immunohistochemistry demonstrated upregulation of eNOS expression up to 7 days, but not 14 days abstention. Expression of iNOS declined at 0 day. These data suggest that chronic cardioprotection against ischemia-reperfusion injury by regular ethanol consumption persists for at least 7 days after abstention. Increased eNOS activity plays a role in this persistent cardioprotection [100].

Decreased expression of iNOS in ethanol treated hearts might be due to attenuation of inflammation. Growing evidence suggests that anti-inflammatory effects of moderate ethanol consumption not only reduce the incidence of coronary artery disease [101] but also attenuate inflammation after ischemia-reperfusion [102]. Previous experiments suggest that inflammation plays a role in ischemia-reperfusion injury [103]. NO derived from iNOS mediates cardiac dysfunction by inducing production of pro-inflammatory cytokines [104]. Cytokines such as IL-1, IL-6, and TNF-$\alpha$ are upregulated rapidly in response to myocardial ischemia [105]. Yamaguchi et al. found that the late phase of ethanol cardiac preconditioning correlated with reduced leukocyte-endothelial cell adhesive interactions, suggesting an anti-inflammatory effect [106]. This effect appears dependent on PKC [107]. eNOS was shown to be essential for the anti-inflammatory effects of the late phase of ethanol preconditioning [108].

Recently, prevention of mitochondrial permeability transition pore (mPTP) opening by inhibition of glycogen synthase kinase 3β (GSK-3β) has been implicated in ischemic preconditioning [109]. Irreversible mPTP opening abolishes the mitochondrial membrane potential, disabling the mitochondria ATP production. GSK-3β is a substrate of multiple pro-survival protein kinases, including Akt, ε-PKC, extracellular signal-regulated kinase 1/2 (ERK) and protein kinase G. Inhibition through phosphorylation of GSK-3β by PI3/Akt

signaling is a key event for prevention of mPTP opening, increasing cell survival [110]. Zhong et al. reported that mice fed 18% ethanol in their drinking water for 12 weeks demonstrated sustained translocation of ε-PKC and increased expression of Akt [111]. Recently, Zhou et al. demonstrated that 10-500 μM (light) but not 1 mM (heavy) ethanol prevents mPTP opening by inhibiting GSK-3β in cardiac H9c2 cells [112]. GSK-3β has also been shown to play a role in apoptosis [113]. Active GSK-3β facilitates apoptosis induced through mitochondrial pathway, whereas it suppresses pro-apoptotic signaling from death receptors such as Fas, TNF-R1 and DR4,5 [113-115]. It remains unclear whether chronic alcohol consumption affects mitochondrial apoptotic protein, such as Bax, Bad and Bcl2 by modulating GSK-3β. Further studies are needed. As GSK-3β plays an important role in cardiomyocyte death, manipulation of this protein kinase by ethanol may help in developing strategy for cardioprotection. The role of other protein kinases such as protein tyrosine kinase and mitogen-activated protein kinase (MAPK) in cardioprotection by regular ethanol consumption has not been established. Both kinases have been implicated in ischemic preconditioning [116-118]. A study demonstrated that the infarct size limiting effect of acute ethanol exposure was abolished by genistein (non-selective protein tyrosine kinase) in isolated rabbit hearts [119]. MAPK such as p38 MAPK, ERK and c-Jun NH2-terminal (JNK) have been also implicated in ischemic preconditioning [120]. Sanada et al. reported that transient p38 MAPK activation during preconditioning is a trigger for IPC [118]. Involvement of ERK and JNK in cardioprotection by ethanol remains unclear. One study demonstrated that acute moderate ethanol exposure (4 or 8 mM), but not heavy exposure, to vascular smooth muscle stimulated ERK activity which was blocked by PKC inhibitor [121]. In contrast, Churchill et al. reported that intraperitoneal injection of ethanol (0.5g/kg) in mice 60 min prior to ischemia-reperfusion reduced phosphorylation of ERK and JNK compared with

control hearts [78]. Further study will be needed to elucidate involvement of ERK and JNK in cardioprotection by regular ethanol consumption.

## Cardioprotection by Chronic Ethanol Consumption: dose, duration and washout

A cardioprotection by regular ethanol consumption is dependent on the time and duration of ethanol exposure. The definition of one ethanol drink varies remarkably by publication and countries. The terms such as light, moderate and heavy drinking are vague. For example, one drink is 0.5 fl oz or 12 g of ethanol in USA, 8 g in England and 20-24g in Japan. According to *Dietary Guidelines for Americans*, moderate drinking is no more than 1 drink (12 g of ethanol) per day for women and no more than 2 drinks (24 g of ethanol) per day for men [122]. In experimental studies, the concentration of ethanol varies significantly. There are differences in dose and duration of ethanol consumption, mode of administration, units used to express the amount of ethanol (mg/dl, mM etc.). Serum levels of ethanol fluctuate depending on the time of blood sampling as animals do not drink constantly through the day. Further, elimination rates of ethanol are different among species. For example, guinea pigs eliminate ethanol 50 to 60 % faster (2.59 micromol/g liver/min) [123] than humans (1.63-1.76 micromol/g liver/min) [124]. We fed guinea pigs a nutritionally supplemented liquid diet containing 15% ethanol-derived calories for 8 weeks [58]. This approximates the upper limits of moderate ethanol consumption in human (45 g of ethanol/day, at 7 kcal/g ethanol, results in ~15% ethanol-derived calories in a 2000 kcal diet) [125]. In this study, serum ethanol levels at 9-11am were 10±2 mg/dl (about 2.2 mM). The duration of treatment is also important. In our early studies of ethanol preconditioning,

the duration required for cardioprotection was at least for 3 weeks with 10% ethanol in the drinking water of guinea pig. Treatment for 3 weeks with 2.5% or 5% ethanol did not induce full protection [17]. Rat hearts treated with ethanol dosed at 9%(v/v) for 7 weeks [60] or 18% (v/v) for 10 months [81] both showed a cardioprotection after ischemia-reperfusion, with average serum ethanol levels of 1.7±0.1 mg/dl (about 0.4 mM) or 15.4±0.6 mg/dl (about 3 mM), respectively. Pagel et al. fed dogs with ethanol (1.5g/day) mixed with dry food twice per day for 12 weeks. Average serum ethanol concentration was 11 mM [61]. Chen et al. used 10 mM ethanol to induce cardioprotection by acute ethanol exposure in isolated perfused rat hearts before and throughout ischemia [126]. However, the presence of ethanol during ischemia seems to have antiprotective effects in rabbit hearts. Krenz et al. demonstrated that ethanol levels of between 6 and 12 mM during ischemia abolished the protective effect of acute ethanol exposure in *in vivo* rabbit hearts [127]. They also found that ethanol at concentrations of 5-50 mM reduced infarct size if washed out before ischemia. They determined that a threshold ethanol level for cardioprotection in rabbit was approximately 3 mM. In previous studies from our laboratory, whether ethanol was withdrawn 12-16 h before ischemia (wash out) or ethanol was not withdrawn from the drinking water before sacrifice (serum ethanol levels at the time of sacrifice were 2.7±0.5 mM in guinea pigs receiving 2.5% ethanol), cardioprotection from ischemia-reperfusion injury was observed [17,99].

Thus, the serum ethanol levels can vary remarkably depending on dosing, time of blood sampling, and species.

## Clinical Perspective

Data presented in this review would suggest that ethanol can be used as a potential cardioprotective therapeutic agent. However, the negative effects of excessive drinking must be taken into account. Heavy drinking is one of the leading preventable causes of death worldwide [128]. A recent study has shown that one in every 25 deaths worldwide and 5% of global disability-adjusted life-years are attributable to ethanol consumption [129]. Heavy drinkers have a much higher rate of morbidity and mortality that overcomes any potential benefit against ischemic heart disease. Yet, epidemiological evidence suggests that moderate drinking reduces the risk of ischemic heart disease. Further, animal data suggest that regular ethanol consumption protects against ischemia-reperfusion injury; a possible mechanism accounting for increased survival after myocardial infarction in moderate drinkers versus abstainers. And as stated above, we found that the infarct size limiting effect by regular ethanol consumption persists for at least 7 days after abstention. Nevertheless, it would be inappropriate to recommend drinking to patients to protect their heart. While ethanol itself cannot be recommended as a therapeutic agent, understanding the mechanisms underlying its cardioprotective effects could lead to safer therapies.

It is important to note that recent studies demonstrate acute (as opposed to chronic) ethanol exposure fails to exert a cardioprotective effect during ischemia-reperfusion [127]. A recent randomized, prospective study in human found that administration of a moderate dose of ethanol abolished ischemic preconditioning and was associated with worsening subsequent ischemia [130].

Elucidating the mechanism underlying ethanol cardioprotection may help in the development of cardioprotective therapies in the perioperative setting. We recently found

that the infarct size limiting effect by regular ethanol consumption is enhanced by sevoflurane [99]. Because anesthetics can be administrated with relatively low toxicity, elucidating the interaction between ethanol and volatile anesthetic-induced preconditioning and the underlying mechanisms may be beneficial for reducing perioperative myocardial ischemia-reperfusion injury. Growing evidence that long-term ethanol consumption mimics ischemic preconditioning opens a new avenue for developing novel therapies to improve outcomes in patient at risk for myocardial infarction.

## ACKNOWLEDGEMENTS

This study was supported by Grant-in-Aid for Scientific Research (C) 20592382 from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Masami Miyamae) (Tokyo, Japan), "High-Tech Research Center" Project for Private Universities: matching fund subsidy, 2007-2011 from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Naochika Domae) (Tokyo, Japan).

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## Figure Legends

Figure 1: Dose effect and potential mechanisms for decreasing incidence of coronary heart disease by regular ethanol consumption

TNF-R1, R2=tumor necrosis factor-receptor 1, 2

Figure2. Hypothetical scheme for cardioprotection by ethanol against myocardial ischemia-reperfusion injury

PLC=phospholipase C; PLD=phospholipase D; PKC=protein kinase C; PI3K=phosphoinositide 3-kinase; Akt=protein kinase B; GSK3β= glycogen synthase kinase 3β; pGSK3β=phospho glycogen synthase kinase 3β; PTK=protein tyrosine kinase; ROS= reactive oxygen species; JNK= c-Jun N-terminal kinase; ERK= extracellular signal-regulated kinase; MAPK= mitogen-activated protein kinase; ALDH2= aldehyde dehydrogenase 2; 4-HNE=4-hydroxy-2-nonenal; Bcl2=B-cell leukemia 2 family protein; Bad=Bcl2-antagonist of cell death; mPTP=mitochondrial permeability transition pore; NO=nitric oxide; eNOS=endothelial nitric oxide synthase.

Dotted line; to be elucidated.

## Table Legends

Table 1; Isolated guinea pig hearts were subjected to 45 min of global ischemia and 48 min of reperfusion. Experiments were performed in hearts from guinea pigs consuming varying concentrations of ethanol in their drinking water for 3, 6, or 12 weeks. A group of hearts from animals not exposed to ethanol was subjected to ischemic preconditioning (PC). PC was elicited by 2 min of global ischemia and 5 min of reperfusion immediately before 45 min of ischemia. Creatine kinase release was measured in the coronary effluent during first 18 min of reperfusion (3-min intervals). Data are presented as mean ± SEM.

Table 2; Effect of acute and chronic ethanol exposure to hearts against ischemia-reperfusion injury