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# Forum Review

# Ethanol, Wine, and Experimental Cardioprotection in Ischemia/Reperfusion: Role of the Prooxidant/Antioxidant Balance

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## ABSTRACT

**It is now well established that oxidative stress resulting from reactive oxygen species (ROS) that are generated in cardiac myocytes subjected to ischemia/reperfusion plays a causative role in the development of heart failure and may contribute to promote cell death. During the last decade, several groups have reported that, in animal models of myocardial ischemia/reperfusion, certain nutrients, including ethanol and nonethanolic components of wine, may have a specific protective effect on the myocardium, independent of the classical risk factors implicated in vascular atherosclerosis and thrombosis. Mechanisms through which the consumption of alcoholic beverages protects against ischemia-induced cardiac injury are still unknown. One major open question is whether ethanol and nonethanolic components of wine are cardioprotective, at least in part, by interfering with the myocardial prooxidant/antioxidant balance. Important concepts, such as cardiac preconditioning, are now entering the field of nutrition, and recent experimental evidence suggests that ethanol and/or nonethanolic components of wine might exert preconditioning effects in animal models of myocardial ischemia/reperfusion. There is no doubt that such an observation, if confirmed in human subjects, might open new perspectives in the prevention and treatment of ischemic coronary heart disease.** *Antioxid. Redox Signal.* 6, 431–438.

## INTRODUCTION

CARDIOVASCULAR DISEASE, in particular ischemic heart disease, remains a major contributor to morbidity and mortality in developed and developing countries. Ischemic heart disease includes a number of clinical entities, such as transient angina pectoris of effort, unstable angina, silent ischemia, stunning and hibernation, and the more sustained pattern of ischemic ventricular dysfunction in acute myocardial infarction and ischemic cardiomyopathy, one of the end results of severe chronic coronary artery disease (51). Myocardial ischemia may occur as a result of atherosclerotic lesions of the coronary arteries or may be secondary to platelet aggregation and/or coronary artery spasm. It is generally associated with a marked decrease of the normal cardiac function and severe metabolic changes. It has been well established that, in patients suffering acute myocardial infarction (AMI), early coronary reperfusion by means of coronary bypass surgery, transluminal angioplasty, or thrombolytic therapy is the only way to limit irreversible tissue injury and infarct size (27). However, experimental studies designed to investigate the consequences of restoring blood flow to the ischemic myocardium have shown that reperfusion may by itself increase the apparent severity of tissue injury, a phenomenon known as "reperfusion injury." This includes arrhythmias and a prolonged, but fully reversible, mechanical dysfunction (stunning).

It is now generally accepted that oxidative stress induced by reactive oxygen species (ROS) plays an important role in

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the pathogenesis of ischemia/reperfusion. Indirect evidence for a role of oxyradicals in ischemia/reperfusion-induced myocardial damage comes in particular from experiments showing a protective effect of antioxidants (2, 57). This concept may have significant therapeutic implications because it suggests that antioxidant therapies begun after the onset of ischemia could be effective in preventing postischemic dysfunction.

Both epidemiological and experimental studies indicate that mild-to-moderate consumption of alcoholic beverages, in particular red wine, may have beneficial effects on the heart, and that moderate drinkers are at low risk for coronary heart disease, the most common form of heart disease, compared with heavy drinkers or abstainers (8, 22, 23, 33, 53, 59, 63, 69, 72).

Considerable controversy has arisen regarding the mechanisms that may be responsible for alcohol-induced cardiac protection, and several different mechanisms have been suggested. These include an effect on lipoprotein oxidation and favorable alterations of blood chemistry leading to the prevention of clot formation in coronary arteries, with a decreased rate of atherosclerotic and thrombotic coronary artery obstruction (12, 58, 64). However, other mechanisms are probably involved. For instance, recent studies have shown that moderate drinking may improve early outcome after AMI and prevent sudden cardiac death (1, 14, 46, 47), suggesting a direct protective effect on the myocardium.

On the other hand, besides the cardioprotective effect of ethanol itself, the antioxidant properties of nonethanolic compounds abundantly present in some alcoholic beverages (for instance, in red wines compared with white wine, beer, or spirits) may be involved in the protection resulting from their consumption (11, 65).

The main objectives of this short review are to present the specific effects of ethanol and nonethanolic components of alcoholic beverages in experimental models of myocardial ischemia/reperfusion, and to discuss the possible involvement of alterations in the prooxidant/antioxidant balance in the mechanisms of ethanol-induced cardioprotection.

## CARDIOPROTECTIVE EFFECTS OF ETHANOL

Numerous epidemiological studies have shown that moderate ethanol consumption reduces coronary heart disease (8, 22, 23, 33, 53, 59, 63, 69, 72). Animal models of chronic ethanol feeding have confirmed the cardiovascular benefits observed in human studies (41–43, 52, 76, 77). Thus, in rats and guinea pigs, a sustained (6 weeks) low to moderate ethanol consumption [2.5–36% (vol/vol) in drinking water] resulted in a significant reduction in ischemia/reperfusion-induced injury as evidenced, in *ex vivo* heart preparations, by improved contractile recovery and decreased creatine kinase release (42). The results of this study suggest that regular ethanol consumption induces long-term protection against ischemia/reperfusion injury, which is sustained for at least 18 h after ethanol exposure. However, in rats, long-term moderate consumption of ethanol [15–36% (vol/vol) in dietary water for 16 weeks] failed to alter infarct size compared with control (Table 1) (16).

A 20-min exposure to low doses of ethanol (10 m*M*) also induced cardioprotective effects against ischemia/reperfusion in *ex vivo* isolated heart preparations submitted to 45 min of no-flow ischemia followed by 30 min of reperfusion (9). Significant limitation of infarct size was reported in isolated buffer-perfused rabbit hearts exposed to 10–50 m*M* ethanol for

TABLE 1. SPECIFIC EFFECTS OF ETHANOL AND NONETHANOLIC COMPOUNDS OF RED WINE IN EXPERIMENTAL MODELS OF MYOCARDIAL ISCHEMIA

| *Type of ischemia* | *Treatment* | *Improvement in post-ischemic recovery of ventricular function (% vs. untreated group)* | | *Decrease in infarct size (% of risk area vs. untreated group)* | *MDA (taken as an index of oxidative stress)* |
|---|---|---|---|---|---|
| | | *AF* | *LVDevP* | | |
| GI 30 min/R 2 h in isolated rat heart preparations | RWE 1 μg/ml* | ↑ 55.8% | ↑ 58% | −37,5% | ↓ 33.6% |
| | RVT 10 μ*M** | ↑ 36.39% | ↑ 39% | −67.2% | ↓ 38.8% |
| | Ethanol 0.07%* | No effect | No effect | No effect | No effect |
| | RVT 10 μ*M* + Ethanol 0.07%* | No effect | No effect | −34.5% | No effect |
| RI 30 min/R 2h in isolated rabbit heart preparations | Ethanol 0.7 g/kg (i.v. injection 30 min before the onset of ischemia) | ND | ND | −67% | ND |
| *In vivo* RI 60 min/R 3 h in rats | Ethanol 15% (vol/vol) in drinking water for 16 weeks | No effect | No effect | No effect | ND |
| | Ethanol 36% (vol/vol) in drinking water for 16 weeks | No effect | No effect | No effect | ND |

Data are adapted from references 16, 35, and 66.

GI, global ischemia; RI, regional ischemia (coronary artery ligation); R, reperfusion; RWE, red wine extract; RVT, resveratrol; AF, aortic flow (at 60 min of reperfusion); LVDevP, left ventricular developed pressure (at 60 min of reperfusion); MDA, malondialdehyde level in coronary effluents at 3 min of reperfusion versus untreated group; ND, no data.

*Added to the perfusion buffer 15 min before the onset of ischemia.

5–45 min followed by 10 min of washout before the induction of regional ischemia (30 min) followed by 2 h of reperfusion (Table 2) (35). However, when ethanol exposure was prolonged until the end of ischemia, protection was abolished (35).

Acute exposure to ethanol was also shown to induce protection in isolated cardiac myocytes or intact heart preparations (9, 34, 35). However, a 10-min intravenous ethanol infusion at 0.35, 0.7, or 1.4 g/kg, starting 20 min prior to the onset of ischemia, did not reduce infarct size in *in situ* rabbit hearts. But if ethanol exposure was followed by washout or sufficient time to metabolize the ethanol prior to ischemia, protection was observed (Table 2) (35).

Notable differences between these experimental studies make any comparison difficult. Indeed, there are marked differences in duration and type of ischemia, doses of ethanol, mode of administration, and units used to express the amount of ethanol.

The current recommendation for moderate daily consumption (no more than two drinks per day) roughly corresponds to 10 m*M* blood ethanol level 1 h after consumption. This approximates the upper limits of moderate ethanol consumption in humans (<45 g of ethanol per day) (43) and corresponds to ~15% of the total daily energy intake. In *in vivo* models, in which animals are exposed to ethanol via their drinking water (15%, vol/vol) for 8–12 weeks, there is a maximal blood ethanol level of ~15–20 m*M* that fluctuates during the day.

However, it is noteworthy that, in anesthetized dogs, acute intravenous administration of ethanol (0.8 g/kg) 10 min prior to 60-min regional ischemia induced by coronary artery ligation did not affect either infarct size or hemodynamic variables (30). At similar concentration (0.5 g/kg), ethanol also failed to reduce infarct size in several different animal models of regional ischemia/reperfusion when it was intravenously injected during or prior to coronary artery ligation (3, 25), whereas higher doses (2 g/kg) significantly reduced infarct size (4). This suggested that the cardioprotective effect of ethanol is dose-dependent.

It must also be noted that chronic exposure to elevated doses of ethanol produces toxic effects. Thus, in humans, chronic heavy ethanol consumption is known to result in alcoholic cardiomyopathy (21). Ethanol feeding of rats has shown that long-term heavy ethanol intake may also result in disorders of myocardial function with decreased myocardial blood flow, increased vascular resistance, and ventricular arrhythmias (45).

Therefore, despite many studies showing that ethanol is able, in certain conditions, to protect the ischemic/reperfused myocardium, the precise cellular mechanisms by which moderate ethanol intake exerts such a beneficial effect still remain to be elucidated.

Recently, it has been reported that 3–12 weeks of moderate to heavy ethanol consumption reduces ischemia/reperfusion injury to the same degree as the acute effect of ischemic preconditioning (IPC) in guinea pig heart (42). In this view, it has been proposed that regular ethanol consumption may protect the heart against ischemia/reperfusion injury by mimicking the classical IPC (48). IPC is a phenomenon in which single or multiple brief periods of ischemia have been shown to protect the heart against a more prolonged ischemic insult, the result of which is a marked reduction in myocardial infarct size, severity of stunning, or incidence of ventricular arrhythmias (48).

All these consequences are potentially important in the clinical setting. Many groups have tried (and continue to do so) to understand the mechanisms of IPC in order to design new pharmacological approaches for the treatment of coronary heart disease. A major goal of cardiovascular research is to determine whether it is possible to induce pharmacologically a chronic state of preconditioning. A clue to achieving this goal may be in the sustained protective effect of regular ethanol consumption against ischemia/reperfusion injury (42). In this view, it must be emphasized that recent clinical evidence suggests that regular ethanol consumption, in addition to decreasing the incidence of AMI (33, 64, 69), may also improve survival after AMI (14).

Recent investigations have reported that chronic moderate ethanol consumption may mimic the protective effect of IPC by signaling pathways that involve adenosine A1 receptors, ATP-sensitive potassium ($K_{ATP}$) channels and protein kinase C (PKC) activation (41–43, 52, 76, 77). However, IPC is a response to an acute stress, whereas the protective effect of ethanol drinking is the consequence of a regular exposure. This raises the question of whether IPC and ethanol preconditioning involve the same signaling pathways. Several investigators have indeed proposed that both ethanol and IPC activate the $\varepsilon$ isoform of PKC ($\varepsilon$-PKC), which could be protective through an effect on $K_{ATP}$ channels (41, 77). It is therefore likely that IPC and ethanol preconditioning share a common pathway. On the other hand, the mechanisms implicated are probably much more complex than previously suspected, and will not be discussed further here. Indeed, other pathways are probably involved including, for instance, the generation of

TABLE 2. EFFECT OF ETHANOL CONCENTRATION ON EXPERIMENTAL MYOCARDIAL INFARCT SIZE

| *Experimental model* | *Treatment* | *Decrease in infarct size (% vs untreated group)* |
|---|---|---|
| *In vivo* CAO 30 min/R 3 h in rabbits | Ethanol 0.35 g/kg (injection) 60 min before the onset of ischemia | −30% |
| | Ethanol 0.35 g/kg 20 min before ischemia | No change |
| CAO 30 min/R 3 h in isolated perfused rabbit heart preparations | Ethanol 50 m*M* (injection) 20 min before the onset of ischemia | −43% |
| | Ethanol 50 m*M* during ischemia | No change |

Data are adapted from reference 35. Infarct size is expressed as % of risk zone versus untreated group. CAO, coronary artery occlusion; R, reperfusion.

nitric oxide (NO) and/or free radicals (9, 34, 35, 41–43, 52, 76, 77), and it is likely that ethanol preconditioning involves biological mechanisms that are not (at least fully) implicated in IPC. Thus, IPC and ethanol preconditioning, even if they share common signaling pathways (in particular PKC activation), are probably two different forms of protection.

## CARDIOPROTECTIVE EFFECTS OF NONETHANOLIC COMPONENTS OF ALCOHOLIC BEVERAGES

According to many authors, the benefits from alcoholic beverage consumption on the incidence of cardiovascular diseases is particularly relevant after the consumption of wine, and more specifically red wine (54, 63, 69). Indeed, red wine contains constituents other than ethanol (for instance, polyphenolic compounds) that could have cardioprotective properties (75).

Several studies support a protective effect of polyphenols against cardiovascular diseases and cancer, although other studies failed to demonstrate any beneficial effect (65, 68). Flavonoids are the most common group of plant polyphenols, including the anthocyanidins, flavones, flavanones, flavonols, isoflavones, and catechins. These compounds possess major antioxidant and free radical scavenging properties that could be involved in their biologic effects (65, 68). However, no randomized clinical trial has ever been designed to date to test these compounds properly in the human setting. They are, however, probably responsible for the antioxidant activity of wine, and the beneficial effect of moderate wine drinking has been attributed, at least in part, to the antioxidants present in the polyphenolic fraction.

It is noteworthy that grape seed proanthocyanidins were actually shown to have cardiac protective effects against reperfusion-induced injury in a rat model of myocardial ischemia via their ability to reduce or remove, directly or indirectly, free radicals produced in myocardium during postischemic reperfusion (53).

Resveratrol (3,5,4′-trihydroxystilbene) is a naturally occurring phytoalexin, present in *cis* and *trans* configuration in some spermatophytes (grapes, eucalyptus, peanuts, and pines), in which its synthesis is induced by stress, injury, or infection (68). It has been shown to inhibit platelet aggregation (55), to alter eicosanoid synthesis (44), and to modulate lipid and lipoprotein metabolism (68). In addition, it was recently shown to inhibit ribonucleotide reductase and DNA synthesis in mammalian cells, with further possible applications as an antiproliferative or a cancer chemopreventive agent in humans (6, 20, 31). Because this compound is abundant in grape berry skins but not in flesh, it is routinely found in red wines (at a concentration ranging between 0.1 and 15 mg/L), but only in very small amount in white wines. In contrast with most flavonoids, it is absent in nonalcoholic beverages because it is not water-soluble. Its presence in nuts (especially peanuts) corroborates the theory (essentially supported by the results of epidemiological studies) that nuts also are cardioprotective (13).

Animal experiments have shown that resveratrol protects the heart from ischemia/reperfusion injury (26). Preconditioning of the heart with resveratrol resulted in improved postischemic ventricular functional recovery and reduced infarct size (Table 1) (26). It was speculated that resveratrol could pharmacologically precondition the heart in a NO-dependent manner. Actually, resveratrol was unable to precondition the inducible NO synthase (iNOS) knockout mouse heart, whereas it could successfully precondition the wild-type mouse hearts, indicating an essential role of iNOS in resveratrol preconditioning of the heart (29). By using cultured endothelial cells from human veins, resveratrol was shown to up-regulate endothelial NO synthase (eNOS) mRNA expression in a time- and concentration-dependent manner (74). Resveratrol also enhanced the production of bioactive NO and increased the activity of the eNOS gene promoter (74). All these data suggest, but do not prove, that resveratrol may protect the heart via an effect on eNOS. In addition, it was shown that a reasonable consumption of certain resveratrol-rich wines results in a significant accumulation of that lipophilic substance in tissues such as heart and kidney (5). Thus, the preconditioning effect of resveratrol may be dependent on its presence *in situ*. It remains to be determined what the molecular target of resveratrol is in the myocardium or in the cardiac cell.

In a recent article, Revel *et al.* (60) proposed that resveratrol is a ligand (with antagonistic abilities) of the aryl hydrocarbon receptor (AHR), a receptor whose activation has been implicated in the toxicity induced by dioxin and other industrial chemicals. Interestingly, the cardiotoxicity of these chemicals is likely related to the presence of the AHR in the heart (28, 73). Although it is rather speculative, this view may open new windows in that complex problem.

Thus, beside its cancer chemopreventive activity (6, 31) and modulation of prostaglandin synthesis (44), resveratrol may be a major cardioprotective nutrient, at least when it is associated with ethanol in wine (Table 1). As underlined by Revel *et al.* (60), resveratrol is indeed a major component of the Mediterranean diet and likely implicated in the French paradox (15).

## CARDIOPROTECTION AND REDOX BALANCE

Heart muscle damage represents one of the consequences of excessive ethanol ingestion (21, 62). It is manifested as a variety of metabolic and function abnormalities, including diastolic dysfunction, reduced ejection fraction, left ventricular hypertrophy, cardiomegaly, and atrial fibrillation (62). It has been suggested that the pathogenic mechanisms responsible for these alterations involve ROS-induced cell injury (49). In fact, ROS and free radicals are generated during ethanol metabolism, causing oxidative stress and lipid peroxidation in cardiac tissue (10), as well as in many other organs, such as liver, brain, and skeletal muscle (36, 49). Furthermore, ethanol metabolism-induced oxidative stress also causes oxidative degradation of the mitochondrial genome (36). Extensive degradation/depletion of mitochondrial DNA followed by recovery was indeed reported to occur in heart, brain, and skeletal muscles of mice 2 h after an acute ethanol administration (5 g/kg) (36). This effect was prevented by vitamin E and melatonin, two powerful antioxidants acting on both ROS and ROS-induced lipid peroxidation (36). Heavy chronic ethanol intake has been

demonstrated to induce marked alterations in the cardiac antioxidant defense system, decreasing cellular reduced glutathione, as well as cytosolic and membranous protein thiols, an effect that could contribute to the increase in free radical formation in the tissue (18, 24, 61). Based on these findings, it has been suggested that changes in the cellular oxidant–antioxidant balance are involved in ethanol-induced cardiotoxicity and that ROS may play an important role in its pathogenesis (Fig. 1). The contribution of ROS to heart disease has been well documented (38), and oxidative stress is generally thought to represent a fundamental mechanism in the induction of various types of myocardial injury (71). The likelihood that ROS-dependent mechanisms might be involved in cardiac injury during ethanol intoxication is supported by a number of experimental and clinical studies (56). In contrast, it must be emphasized that a significant increase in the level of several antioxidant enzymes (*e.g.,* superoxide dismutase, catalase) has been observed in animals under conditions of chronic ethanol administration in liver (50), as well as in cardiac tissue (17, 19, 32). On this basis, it has been hypothesized that chronic ethanol consumption would increase the antioxidant status of the heart and decrease the injury induced to the heart by ischemia and reperfusion (39, 40). These observations might well suggest the existence of an adaptative response to the prooxidative effect of ethanol. Besides, it has been reported that under *in vitro* conditions, ethanol is able to behave as an antioxidant, reducing the formation of lipid peroxidation products in human low-density lipoproteins and delaying the onset of the propagation phase for conjugated dienes and thiobarbituric acid reactive substances (7). This antioxidant effect of ethanol does not appear to be dependent on its concentration, low ethanol concentrations (4.2 m*M*) being able to scavenge hydroxyl radicals (7). Furthermore, due to its ability to induce heat shock protein 70 (HSP 70), ethanol has been shown to improve significantly the cellular antioxidant defenses, an effect that can contribute to cardiac preservation during ischemia/reperfusion (70). Finally, ethanol has been shown to act on several signal transduction mechanisms involved in the inhibition of smooth muscle cell proliferation and migration, and in the activation of the release from vascular cells of vasoactive factors such as NO (37). This might be an explanation for the cardiovascular protection induced by moderate doses of ethanol because NO, besides its effects on vascular tone, has been shown to possess antioxidant and antiaggregatory properties and to inhibit both the proliferation of smooth muscle cells and adhesion of leukocytes.

In a recent and very stimulating study (67), Sato and colleagues concluded that ethanol by itself imparts cardioprotection against ischemia/reperfusion by adapting the heart to oxidative stress. Moderate ethanol consumption would induce a significant amount of oxidative stress to the heart that would be translated into the induction of the expression of several cardioprotective oxidative stress-inducible proteins, including HSP 70. Thus, the beneficial effect of wine, particularly red wine, should be interpreted as the result of two different pathways: (a) the polyphenolic antioxidants present in red wine (*e.g.,* resveratrol and flavonoids), which would provide cardioprotection by their ability to function as *in vivo* antioxidants; and (b) the ethanol-induced oxidative stress, which would activate the induction of various defense mechanisms. In their experiments, feeding rats with polyphenolic antioxidants, as well as ethanol, resulted in the improvement of postischemic ventricular function. Additionally, both wine and ethanol triggered a signal transduction cascade by reducing proapoptotic transcription factors and genes, thereby potentiating an antiddeath signal. This resulted in the reduction of myocardial infarct size and cardiomyocyte apoptosis (66).

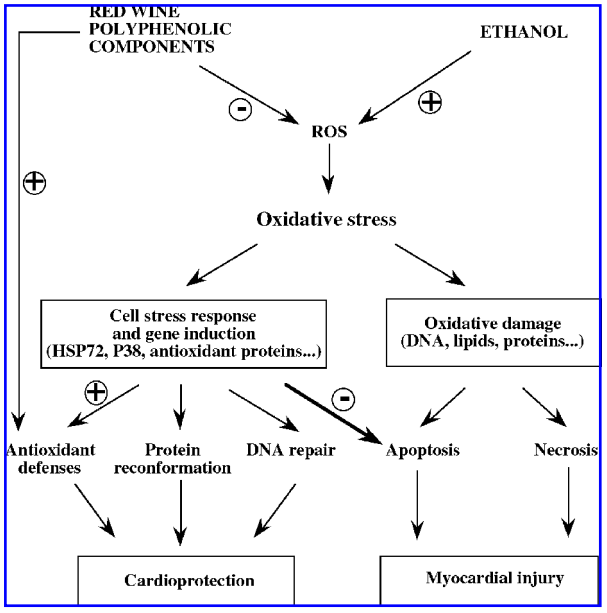


**FIG. 1. Schematic representation of the effects of ethanol and nonethanolic wine components on prooxidant/antioxidant balance.**

## CONCLUDING REMARKS

During the last decade, it has been largely suggested that mild-to-moderate ethanol consumption is associated with a reduced incidence of mortality and morbidity from ischemic heart disease. This cardioprotective effect has been proposed to involve a mechanism similar to that of classical IPC. Despite many attempts to design new pharmacological approaches for the treatment of coronary heart disease over the last 20 years, it is reasonable to say that this has been rather disappointing. No major drug, based on the concept of IPC, has emerged for clinical use. The discovery that chronic moderate consumption of alcoholic beverages may mimic IPC is a great exception even though this protective effect appears to be conditional. However, the precise cellular signaling pathway through which moderate alcoholic beverage intake may exert such a beneficial effect still remains to be elucidated. Growing evidence suggests that the cardioprotective effects of alcoholic beverages would be related to their ability to improve the antioxidant potential of myocardial cells. Epidemiological studies indicate that the consumption of wine, particularly red wine, imparts a greater protection to ischemic heart than does the consumption of other alcoholic beverages. This observation strongly suggests that polyphenolic antioxidants

such as resveratrol and proanthocyanidins could exert cardioprotective effects against ischemia/reperfusion injury by their ability to function as *in vivo* antioxidants. Ethanol contained either in spirits or in wine would then contribute to cardioprotection by adapting the heart to oxidative stress.

## ABBREVIATIONS

AHR, aryl hydrocarbon receptor; AMI, acute myocardial infarction; eNOS, endothelial nitric oxide synthase; HSP, heat shock protein; iNOS, inducible nitric oxide synthase; IPC, ischemic preconditioning; $K_{ATP}$, ATP-sensitive potassium; NO, nitric oxide; PKC, protein kinase C; ROS, reactive oxygen species.

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