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White Wine Induced Cardioprotection against Ischemia-Reperfusion Injury Is Mediated by Life Extending Akt/FOXO3a/NFκB Survival Pathway

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Recent studies on the protection afforded by moderate wine consumption against cardiovascular diseases have focused mainly on the activity of red wine in view of its high content of antioxidants, especially polyphenols. White wine lacks polyphenols, but it contains other compounds such as hydroxycinnamic acids (caffeic acid) and monophenols (tyrosol), which are known to have antioxidant properties. Therefore, this study was designed to examine the effect of white wine in myocardial ischemic-reperfusion injury. The experimental rats were gavaged with white wine (Soave Suavia "Le Rive" 2004) at a dosage of 6.5 mL/(kg·rat·day) for 30 days. Rats were divided into four groups: control sham (CS), wine-treated sham (WS), control ischemia (I)/reperfusion (R) (CIR), and wine + IR (WIR). All the rats in both IR groups underwent 30 min occlusion of the left anterior descending coronary artery followed by 8, 24 h, and 30 days of reperfusion (R). Significant reduction in infarct size (21 vs 39%, $n = 6$), cardiomyocyte (274 vs 384 counts/100 HPF, $n = 6$), and endothelial cell apoptosis (387 vs 587 counts/100 HPF) was observed in WIR as compared with CIR after 24 h of reperfusion. Echocardiography demonstrated significant increased fractional shortening (32 vs 22%) and ejection fraction (60 vs 44%) following 30 days of reperfusion in WIR rats compared to CIR ($n = 6$). In addition, increased phosphorylation of AKT, Foxo3a, and eNOS were found in WS and WIR, as compared to their respective controls. The gel-shift analysis demonstrated significant upregulation of DNA binding activity of NF-κB in the white wine-treated groups. This report demonstrated for the first time that the white wine mediated cardioprotection in ischemic reperfused myocardium is through the PI-3kinase/Akt/FOXO3a/e-NOS/NF-κB survival pathway.

KEYWORDS: White wine; Akt; FOXO3a; eNOS; ventricular modeling

INTRODUCTION

Wine, a natural tranquilizer, has been called the beverage of moderation (*1*). Consumption of wine at moderate doses is found to reduce the risk of coronary heart disease (*2*) The cardioprotective effect of several bioactive compounds is mainly due to the phenolic groups present in their structure. Wine polyphenols have been shown to scavenge peroxynitrite and exhibit antioxidant, anti-inflammatory, and antiatherogenic effects (*3*). The cardioprotective effects of red wine are also thought to be due to catechin-like flavanoids and other polyphenolic compounds such as resveratrol and quercetin. It was reported that grape extract induces cardioprotection by scavenging superoxide and hydroxyl radicals in the ischemia-reperfused myocardium (*7*). Our laboratory have documented that resveratrol treatment in diabetic and hypercholesterolemic rats protected the myocardium from the adverse effects of ischemia-reperfusion injury (*4–6*).

Baur et al. has demonstrated that resveratrol improves the health and survival of mice on a high calorie diet (*8*). Although the cardioprotective effects of red wine are well-demonstrated, white wine research is still in infancy due to its less polyphenolic nature. The active components in white wine include caffeic acid, tyrosol, and shikimic acid. It was also shown that tyrosol is found in wine only after fermentation but not in grapes (*9*). Even though the potential benefits of these components together in white wine are not well-known, their individual effects were known earlier. It was shown that tyrosol, the major olive biophenol, protects Caco-2 cells against oxidized-LDL-induced injury (*10*). In addition, the active compounds of white wine

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tyrosol and caffeic acid were found to modulate oxidative stress and inflammatory reaction (*10*). Klatsky et al. has shown that white wine and red wine reduce the risk of death to the same extent as compared to other alcoholic beverages (*11*), and caffeic acid present in white wine is twice the concentration as of red wine (*12*). The white wine used in our present study contained all these components especially tyrosol and shikimic acid. Moreover, our earlier reports have shown that white wine scavenged the superoxide and hydroxyl radicals in vitro as well as improved the postischemic contractile recovery and reduction in infarct size (*13*).

In human case control studies, red and white wine demonstrated equal effects on fibrinolytic factors (*14*) and collagen-induced platelet aggregation (*15*). In addition, white wine may contain anti-inflammatory protective substances that lessen cytokine release (*16*). But, the effect of white wine on the cell survival phosphatidylinositol kinase-1 (PI-3K) pathway is not known. Akt, the downstream regulator of PI3K is a key regulator in cell survival and metabolic control in different cell types including cardiomyocytes (*17*). Reports suggest that the targets of phospho-Akt are localized in the nucleus and regulate the members of the fork head transcription family such as FOXO1, FOXO3a, and FOXO4 (*18, 19*). In general, FOXOs activate Fas ligands and Bcl-2-like protein Bim, but active/phosphorylated Akt inhibits the FOXO transcription factor activity and thus prevent proapoptotic signaling (*20*), resulting in Akt-mediated cell survival. Nitric oxide plays an important role in cardioprotection against ischemia-reperfusion injury, and NO donors were shown to mimic ischemic preconditioning-like effect (*21–23*). In conjunction with the earlier studies including ours, we designed our present study to investigate the effect of white wine treatment in a myocardial infarction model. We hypothesized that activation of Akt, FOXO3a, eNOS, and redox transcription factor NFκB might be involved in the white wine mediated repair/survival mechanism involved in functional recovery, reduction in infarct size, and cardiomyocyte apoptosis after myocardial infarction.

MATERIALS AND METHODS

Animals. This study was performed in accordance with the principles of laboratory animal care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (Publication No. 85−23, revised 1985). The experimental protocol was examined and approved by the Institutional Animal Care Committee of the Connecticut Health Center (Farmington, CT).

Experimental design. Male Sprague−Dawley rats weighing 250−275 g were used for the study. The rats were randomized into four groups ($n = 24$ in each group): (1) control sham (CS); (2) wine sham (WS); (3) control IR (CIR); and (4) wine IR (WIR). The experimental rats were gavaged with white wine (Suavia "Le Rive" 2004) containing 13% alcohol at a dosage of 6.5 mL/(kg·day) for 30 days. Corresponding controls were given 13% alcohol (presumed to be nontoxic level) dissolved in water (845 μL of absolute alcohol made up to 6.5 mL with water). The chemical constituents of white wine, Suavia "Le Rive" 2004 are provided in **Table 1**. The dose used (6.5 mL/(kg·day)) was based on in vivo dose−response studies performed in our laboratory with 1, 3.5, 6.5, 10, and 20 mL/kg body wt/day. We found that 1 and 3.5 mL/(kg·day) have no effect on cardiac function, whereas 6.5 and 10 mL/kg/day of white wine demonstrated similar cardioprotection. Higher dose (20 mL/(kg·day)) demonstrated toxic effect on liver enzymes (results not shown). Therefore, we selected 6.5 mL/(kg body wt·day) white wine (Suavia "Le Rive" 2004) for our present study.

After the treatment period, all the rats were subjected to 30 min of temporary LAD occlusion followed by 8 h (phosphorylated protein expression profile), 24 h of reperfusion (infract size and cardiomyocyte apoptosis), and 30 days of reperfusion to measure the cardiac functions by echocardiography.

Table 1. Description of Constituents in Soave DOC Classico 2004 (Le Rive) Wine

contituents (SUALIA)	IUPAC name	conc (mg/L)
shikimic acid	(3*R*,4*S*,5*R*)-3,4,5-trihydroxy-1-cyclohexenecarboxylic acid	19.0
hydroxy-tyrosol	4-(2-hydroxyethyl)-1,2-benzenediol	2.69
tyrosol	4-(2-hydroxyethyl)phenol	17.06
vanillic acid	4-hydroxy-3-methoxybenzoic acid	0.99
caffeic acid	3-(3,4-dihydroxyphenyl)-2-propenoic acid	7.15
ferulic acid	(*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid	1.42
p-cumaric acid	3-(4-hydroxyphenyl)prop-2-enoic acid	0.72
quercetin	3,3′,4′,5,7-pentahydroxy-2-phenylchromen-4-one	<0.1

Surgical Procedure. Male Sprague−Dawley rats were anesthetized with ketamine HCl (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.). Cefazolin (25 mg/kg i.p.) was administered as a preoperative antibiotic cover. After tracheotomy and initiation of ventilation (Harvard Apparatus Rodent ventilator: Model 683), the heart was exposed through a left lateral thoracotomy (4th intercostal space). A 6-0 polypropylene suture was passed under the LAD at the level of the left atrial appendage. A 10 mm section of polyethylene tube was placed on top of the LAD to secure the occlusion without damaging the artery. Both ends of the suture were passed through a segment of flared PE160 tubing to form a snare. Ischemia was induced by pulling the snare and clamping the tube with a hemostat for 30 min. After 30 min of ischemia, the snare was released, and the heart was allowed to reperfuse for 8 h, 24 h, and 30 days, depending on the protocol. Reperfusion was readily confirmed by hyperemia over the surface of the previously ischemic-cyanotic segment. After completion of all surgical protocols, the chest wall was closed in layers, as described previously (*24, 25*). After surgery, analgesic buprenorphine (0.1 mg/kg s.c.) was given, and the animals were weaned from the respirator and were then placed on a heating pad for recovery.

Infarct Size Measurement. Infarct size was measured after 24 h of reperfusion. Animals ($n = 6$) from each group were anesthetized and mechanically ventilated, and the chest wall was opened, as described above. The suture left around the LAD was identified and ligated permanently. 50% Unisperse Blue was injected through the right jugular vein, and the infarct size and area of risk were measured, as previously described (*25*), using Scion image software (Scion Corporation).

Determination of Cardiomyocyte and Endothelial Cell Apoptosis. The rat hearts in both control and wine-treated groups were harvested at predetermined times (i.e., after 24 h of reperfusion for paraffin-embedded tissue sectioning). Double-fluorescent immunohistochemical determination of cardiomyocyte and endothelial apoptosis was performed with terminal dUTP nick end labeling (TUNEL) assay on deparafinized, 4 μm thick sections using an in situ cell death fluorescein detection kit (Roche Diagnostics, Mannheim, Germany) followed by antisarcomeric Actin and antivon Willebrand factor for cardiomyocyte and endothelial cell apoptosis, respectively (*25*)

Western Blot Analysis for Phosphorylated Akt, FOXO3a, and eNOS. To quantify the *p*-Akt, *p*-FOXO3a, and *p*-eNOS, a standard SDS/PAGE Western blot technique was performed. Heart tissues from each treatment group were homogenized and suspended (50 mg/mL) in sample buffer (10 mM HEPES, pH 7.3, 11.5% sucrose, 1 mM EDTA, 1 mM EGTA, diisopropylfluorophosphate (DFP), 0.7 mg/mL pepstatin A, 10 mg/mL leupeptin, and 2 mg/mL aprotinin). The cytosolic protein was isolated, and the total protein concentration was determined using a BCA (bicinchoninic acid) protein assay kit (Pierce, Rockville, IL). The cytosolic proteins were run on polyacrylamide electrophoresis gels (SDS-PAGE) typically using 7% for *p*-eNOS (the phosphorylation site is specific to serine-1177) and 10% for *p*-Akt and *p*-FOXO3a (acrylamide/bis ratios). Standard Western blot technique was done, as

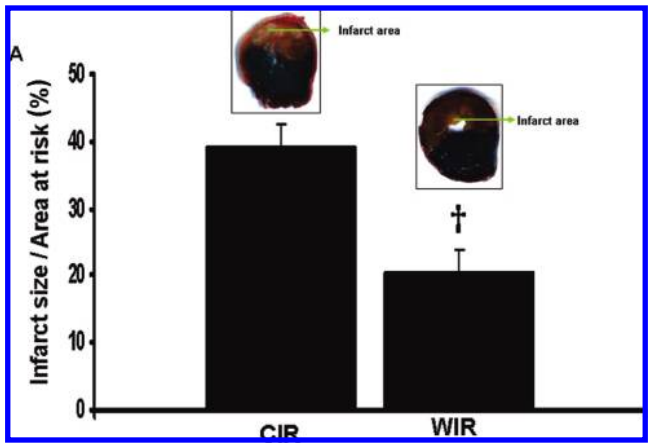

Figure 1. Effect of white wine on infarct size. Representative pictures demonstrate the infarct size in CIR and WIR groups. Graph represents the % of infarct size between the control IR and wine IR groups following 30 min of ischemia and 24 h of reperfusion. Values are mean ± SE ($n = 6$). $^{\dagger}p < 0.05$ represents control IR compared with wine IR group.

described previously (*24*). The antibodies for *p*-eNOS, eNOS, *p*-Akt, Akt, *p*-FOXO3a, and FOXO3a were purchased from Cell Signaling Technology, Danvers, MA.

Electrophoretic Mobility Shift Assay (EMSA). The assay was performed by using 5 μg of the nuclear extracts for 20 min at room temperature with ^{32}P end-labeled oligonucleotides containing the putative NF-κB (5′-AGTTGAGGGGACTTTCCCAGGC-3′) binding site. Reaction products were resolved on 5% nondenaturing polyacrylamide gel. The specificity of the DNA−protein interaction was established by competition experiments using 10 × cold NF-κB oligonucleotide as the competitor. After electrophoresis, gels were dried and visualized by autoradiography (*26*).

Echocardiography. Each rat was sedated using isoflurane (3%, inhaled). When adequately sedated, the rat was secured with tape in the supine position in a custom-built mold designed to maintain the rat's natural body shape after fixation. The hair on the chest wall was removed with a chemical hair remover. Ultrasound gel was spread over the precordial region, and ultrasound biomicroscopy (UBM) (Vevo 770, Visual-Sonics Inc., Toronto, ON, Canada) with a 25 MHz transducer was used to visualize the left ventricle. The left ventricle was analyzed in apical, parasternal long axis, and parasternal short axis views for left ventricular (LV) systolic function, LV cavity diameter, wall thickness, diastolic function, and LV end-systolic and end-diastolic volume determination. 2D directed M-mode images of the LV short axis were taken just below the level of the papillary muscles for analyzing ventricular wall thickness and chamber diameter. All left ventricular parameters were measured according to the modified American Society of Echocardiography-recommended guidelines. Ejection fraction (EF) and fractional shortening (FS) were assessed for left ventricular systolic function. All measurements represent the mean of at least three consecutive cardiac cycles. Throughout the procedure, ECG, respiratory rate, and heart rate were monitored, as described previously (*24, 25*).

Statistical Analysis. Results are expressed as mean ± standard error. ANOVA followed by Bonferroni's correction was carried out to determine any differences between the mean values of all groups. The results were considered significant if $p < 0.05$.

RESULTS

Effect of White Wine on Infarct Size. Hearts subjected to 30 min of ischemia followed by 24 h of reperfusion were used to measure the infarct size. The wine IR group demonstrated significant reduction in myocardial infarct size compared to the control IR group (**Figure 1**). Wine treatment reduced the infarct size approximately to 21% as compared to 39% in the control IR group. The reduced infarct size might be due to activation of the Akt/Foxo3a/eNOS survival pathway.

Effect of White Wine on Extent of Cardiomyocyte and Endothelial Apoptosis. TUNEL assay followed by double antibody staining with anti-α-sarcomeric Actin and antivon Willebrand factor was used to measure the cardiomyocyte and endothelial apoptosis. Results have revealed a significant decrease in the extent of cardiomyocyte apoptosis (274 vs 384 counts/100 HPF) in the wine-treated group ($n = 6$) compared to the untreated group (**Figure 2A**). Similarly, reduced endothelial apoptosis was also found in the WIR group (387 vs 587 counts/100 HPF) as compared to the control IR group (**Figure 2B**).

Effect of White Wine on Myocardial Functions by Echocardiography. Figure 3A shows the representative M-mode images from the CS, WS, CIR, and WIR groups after 30 days of reperfusion. The hearts from sham-operated and white wine-treated rats exhibited a smaller LV cavity and thicker infarct wall. Echocardiographic findings of ventricular performance were similar in the WS and CS groups ($n = 6$). The ejection fraction (60 vs 44%) (**Figure 3B**) and fractional shortening (32 vs 22%) (**Figure 3C**) of the left ventricle were significantly increased in the WIR compared to the CIR group ($n = 6$). The left ventricular chamber was dilated in the CIR compared to WIR as assessed by measuring LVEDD and LVESD. There was a compensatory increase in the posterior (LVPW) and lateral wall systolic thickness in the wine-treated group as compared to the CIR. No significant difference was observed in the left ventricular anterior wall (LVAW) between the WIR and CIR. There were no significant differences in heart rate at baseline or post-MI between the groups (results not shown). On the whole, white wine treatment has demonstrated progressive, significant increase in left ventricular function as compared to the untreated control group.

Effect of White Wine on Phosphorylation of Akt, eNOS, and FOXO3a. The phosphoryaltion status of Akt, eNOS, and FOXO3a proteins was observed after 8 h of reperfusion. White wine treatment has shown significant increase in the *p*-Akt both in the WS and WIR groups (1.5- and 2.6-fold) as compared to the corresponding CS and CIR groups. Similarly, white wine treatment has shown significant increase in the *p*-eNOS level both in the WS and WIR groups (4.2- and 5-fold) as compared to the corresponding CS and CIR groups. The *p*-FOXO3a levels in WS and WIR were also found to be increased as compared to corresponding controls. No significant difference was observed in the nonphosphorylated protein levels of these proteins (**Figures 4** and **5**). The increase in the activation of these survival proteins might have resulted in decreased apoptosis, infarct size, and increased myocardial functions.

Effect of White Wine on NFκB DNA Binding Activity by Gel Shift Analysis. DNA binding activity of NFκB was significantly increased in the white wine sham group as compared to the control sham group. A similar pattern was observed in the WIR group as compared to the control group following IR injury. Significant increase in the DNA binding activity of NFκB was observed on wine treatment as compared to the control IR (**Figure 6**).

DISCUSSION

In this report, we documented for the first time that, like red wine, white wine treatment also renders cardioprotection against myocardial infarction. White wine treatment reduced the infarct size, cardiomyocyte apoptosis, and increased the myocardial functions after ischemia reperfusion as compared to nontreated animals.

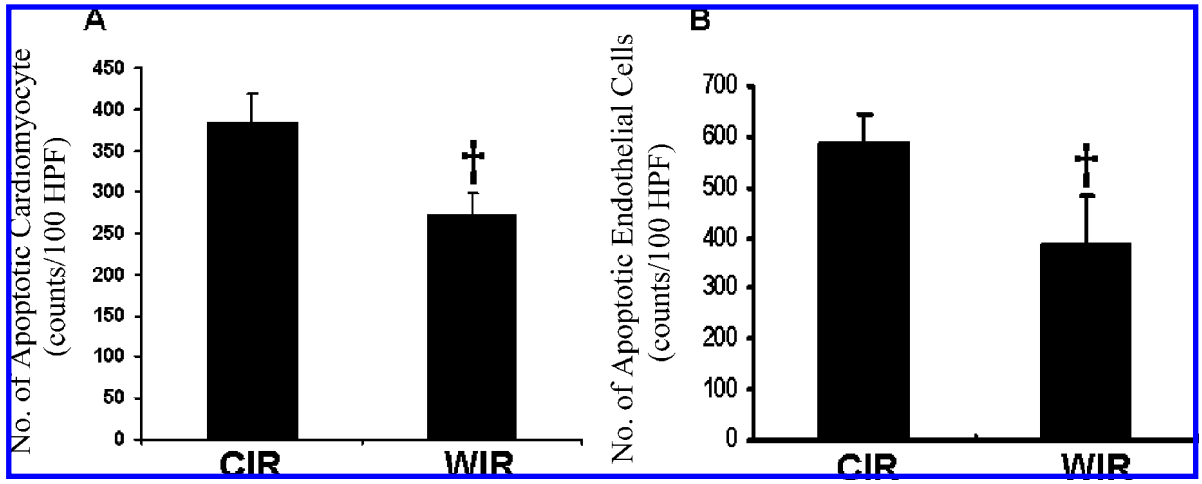

Figure 2. Effect of white wine on cardiomyocyte and endothelial apoptosis. (**A**) Graph represents the difference in number of apoptotic cardiomyocytes between the control IR and wine IR groups following 30 min of ischemia and 24 h of reperfusion. (**B**) Graph represents the difference in number of endothelial apoptosis between the control IR and wine IR groups following 30 min of ischemia and 24 h of reperfusion. Values are mean ± SE (n = 6). $^{\dagger}p < 0.05$ represents control IR compared with wine IR group.

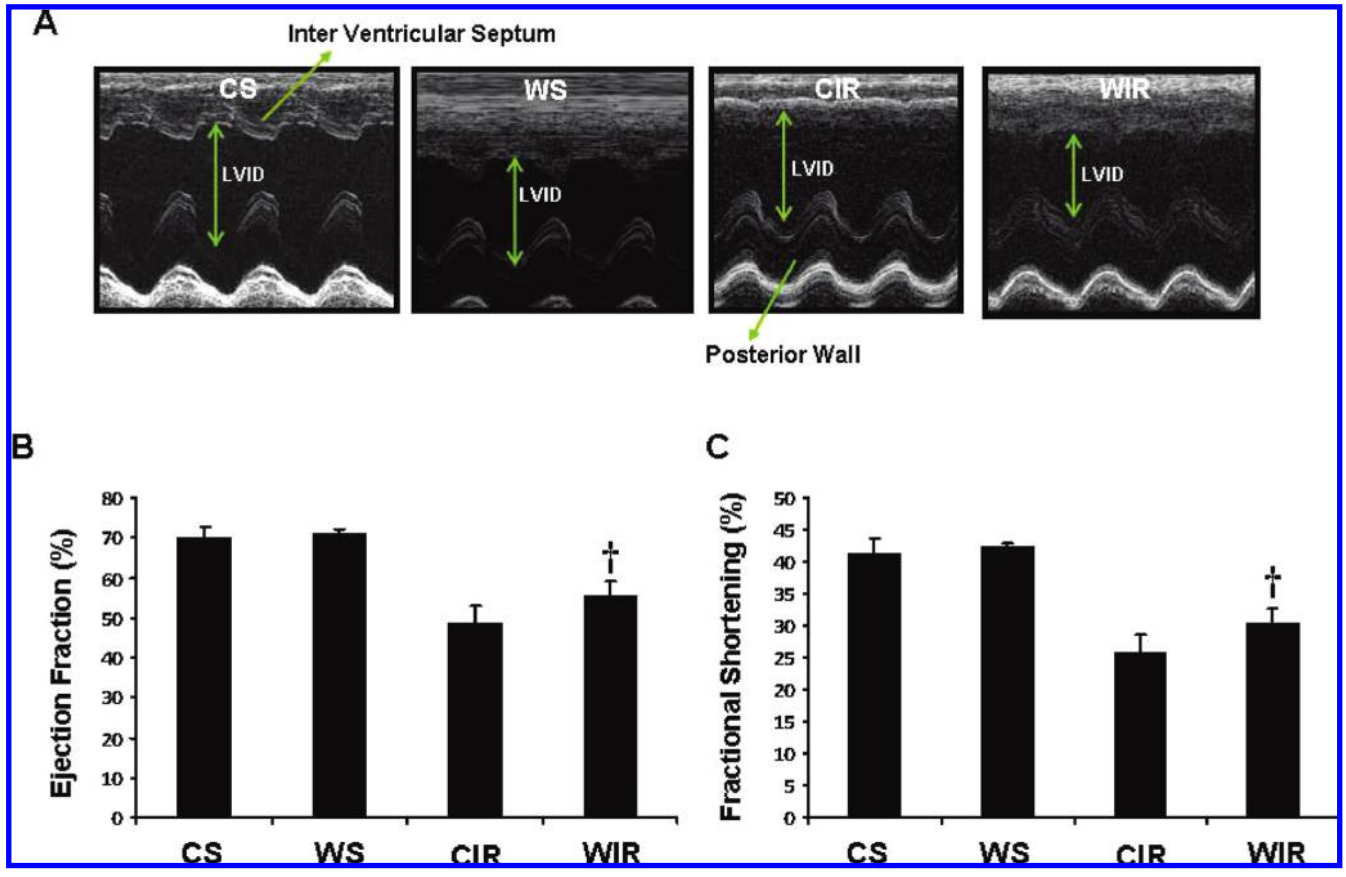

Figure 3. Effect of white wine on cardiac functions by echocardiography. (**A**) Shows the representative pictures in M-mode between the groups. (**B**) Graph represents the % ejection fraction in CS, WS, CIR, and WIR following 30 min of ischemia and 30 days of reperfusion. (**C**) Graph represents the % fractional shortening in CS, WS, CIR, and WIR following 30 min of ischemia and 30 days of reperfusion. Values are mean ± SE (n = 6). $^{\dagger}p < 0.05$ represents control IR compared with wine IR group. CS represents control sham group, WS represents wine sham group, CIR represents control IR group, and WIR represents wine IR group.

The extent of Akt, FOXO3a, and eNOS phosphorylation was also examined in the white wine-treated group. Wine treatment has also shown significant increased phosphorylation of these proteins compared to corresponding controls. The decrease in cardiomyocyte apoptosis and reduction in infarct size on wine treatment might be due to activation of Akt and phosphorylation of FOXO3a. It is demonstrated earlier that activation of Akt is sufficient to inhibit cardiomyocyte apoptosis and also to preserve function in surviving cardiomyocytes (*27*). It is also shown that ischemic preconditioning mediated cardioprotection against ischemia-reperfusion injury is by activation of prosurvival kinases such as Akt during reperfusion (*28*) Adenoviral expression of Akt was found to reduce hypoxia induced cardiomyocyte apoptosis in vitro and in vivo along with reduction in infarct size after transient ischemia (*27*). In addition, phosphorylation of FOXO3a by Akt induces nuclear exclusion of FOXO3a and

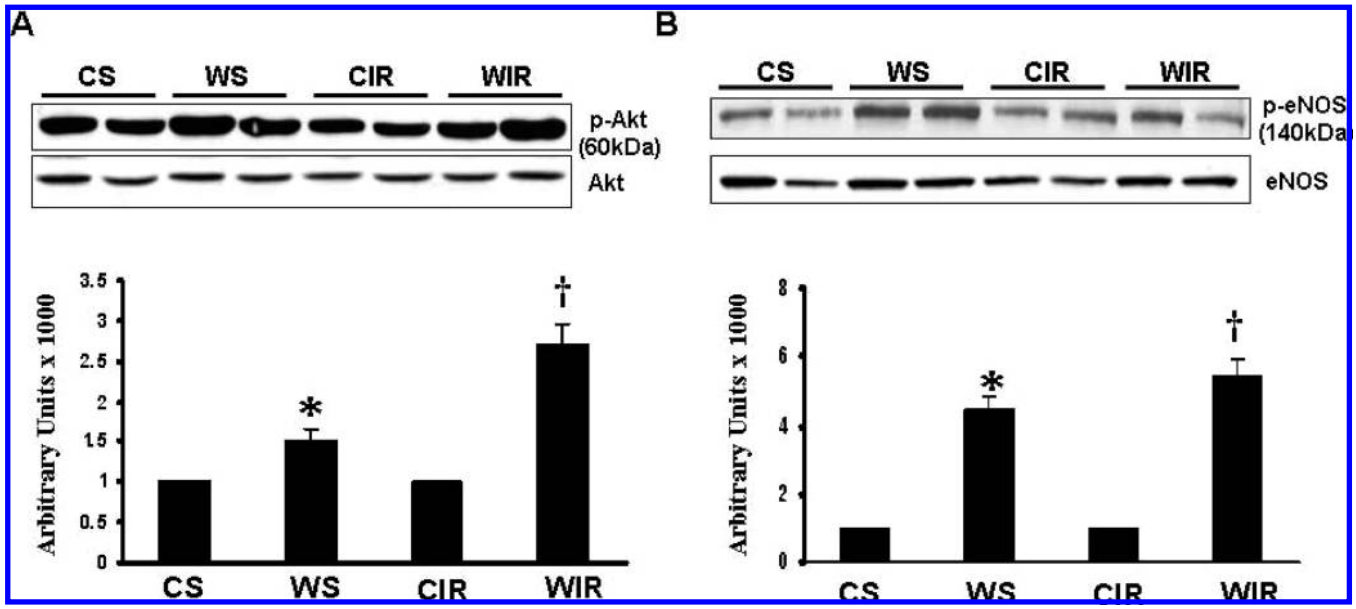

Figure 4. Representative Western blots showing the protein expression of phosphorylated Akt and eNOS. Graph represents the quantitative comparison between the groups. Akt and eNOS were used as the loading control. *$p < 0.05$ represents wine sham compared with control sham group; †$p < 0.05$ represents wine IR compared with control IR group. CS represents control sham group, WS represents wine sham group, CIR represents control IR group, and WIR represents wine IR group.

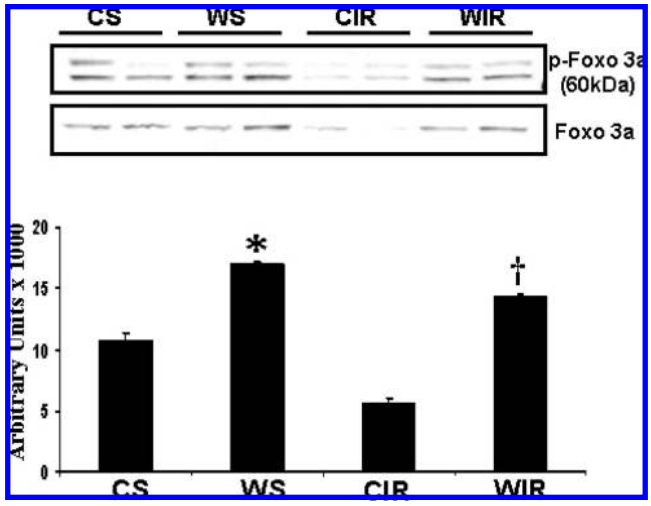

Figure 5. Representative Western blots showing the protein expression of phosphorylated Foxo3a. Graph represents the quantitative comparison between the groups. Foxo3a was used as the loading control. *$p < 0.05$ represents wine sham compared with control sham group; †$p < 0.05$ represents wine IR compared with control IR group. CS represents control sham group, WS represents wine sham group, CIR represents control IR group, and WIR represents wine IR group.

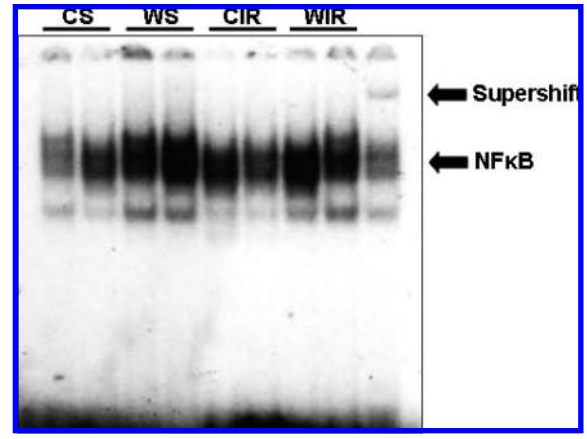

Figure 6. Representative gel shift analysis showing the expression of NFκB. CS represents control sham group, WS represents wine sham group, CIR represents control IR group, and WIR represents wine IR group.

reduces transcription of specific proapoptotic molecules (*29*). Nonphosphorylated FOXO transcription factors have been associated with the activation of several proapoptotic genes, which activates apoptosis. Recently, we have demonstrated that Bromelain induces cardioprotection against ischemia-reperfusion injury through the Akt/FOXO3a pathway in rat myocardium (*30*). In our present study, phosphorylation of FOXO3a by Akt might have resulted in the exclusion of FOXO from the nucleus resulting in reduced cardiomyocyte apoptosis and infarct size.

Dimmeler et al. have shown that phosphorylation and activation of Akt result in the activation of eNOS in endothelial cells (*31*). Several other reports demonstrated the cardioprotective effect of eNOS/NO activation that reduces cardiomyocyte apoptosis and infarct size in a rat model of ischemia-reperfusion injury (*32–34*). Hambrecht et al. have documented the clinical relevance of the activation of Akt and eNOS by demonstrating that regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase (*35*).

Moreover gel-shift analysis has demonstrated increased expression of the transcription factor NFκB expression in white wine-treated groups as compared to corresponding controls that also might have resulted in decreased cardiomyocyte apoptosis as these family of transcription factors are shown to be a direct Akt substrate that mediates a prosurvival effect (*36*). In addition, cytokines such as cardiotrophin-1 that activate Akt have been reported to require activation of NFκB for cytoprotection in cardiomyocytes (*37*). Nitric oxide is also shown to regulate NFκB. Earlier reports have shown that NO can inhibit NFκB activity by several mechanisms (*38, 39*), but Grumbach et al. have demonstrated that, in conditions where eNOS levels are low, concomitant reduction in NO production occurs resulting in NFκB activation and when NO levels are high NFκB levels are inhibited (*40*). It is also shown that NO promotes NFκB

activation in the heart and plays an essential role in the late phase of ischemic preconditioning in rabbits (*41*). Thus, the reduction in infarct size and cardiomyocyte apoptosis on white wine treatment might be due to the activation of Akt and eNOS. Echocardiography also revealed a significant reduction in ventricular remodeling 30 days after I/R in white wine-treated rats as compared to nontreated rats. Significant improvement in ejection fraction and fractional shortening during systole was observed in the white wine-treated group, which demonstrates the efficacy of white wine in maintaining the global left ventricular systolic function.

The increased functional recovery after I/R in white wine-treated rats might be due to increased activation of Akt as it was demonstrated earlier that Akt activation not only reduced cardiomyocyte and endothelial cell death in vivo but also improved regional and overall cardiac function (*27*). Mangi et al. have demonstrated that mesenchymal cells modified with Akt prevent remodeling and restore performance of infarcted hearts (*42*) thus demonstrating the role of Akt in cardioprotection. The increase in cardiac functions and reduction in infarct size and cardiomyocyte apoptosis might also be due to the eNOS activation. It is known that inhibition of eNOS activation and NO production results in impaired endothelial-dependent vasodilation and ventricular function. eNOS-deficient mice were found to develop left ventricular dysfunction and remodeling whereas eNOS-overexpressed mice have been shown to attenuate left ventricular function (*43, 44*). Endothelial nitric oxide synthase gene transfer was found to induce myocardial angiogenesis, reducing cardiac apoptosis after myocardial infarction injury (*45*). Thus, decreased myocardial infarct size, cardiomyocyte apoptosis, and increased myocardial functions on white wine treatment might be due to the activation of Akt/eNOS.

In conclusion, our results demonstrated significant activation of the survival pathway, Akt/FOXO3a/eNOS, by white wine, which can repair infarcted myocardium, prevent ventricular remodeling after myocardial infarction, and establish white wine-based therapy as an effective treatment for human cardiac disease.

LITERATURE CITED

(1) Stockwell, T. R.; Lang, E.; Lewis, P. N. Is wine the drink of moderation. *Med. J. Aust.* **1995**, *162* (11), 578–580.

(2) Di Castelnuovo, A.; Rotondo, S.; Iacoviello, L.; Donati, M. B.; De Gaetano, G. Meta-analysis of wine and beer consumption in relation to vascular risk. *Circulation* **2002**, *105* (24), 2836–2844.

(3) Frohlich, J. J. Effects of alcohol on plasma lipoprotein metabolism. *Clin. Chim. Acta* **1996**, *246* (1−2), 39–49.

(4) Ray, P. S.; Maulik, G.; Cordis, G. A.; Bertelli, A. A.; Bertelli, A.; Das, D. K. The red wine antioxidant resveratrol protects isolated rat hearts from ischemia reperfusion injury. *Free Radical Biol. Med.* **1999**, *27* (1−2), 160–169.

(5) Penumathsa, S. V.; Thirunavukkarasu, M.; Koneru, S.; Juhasz, B.; Zhan, L.; Pant, R.; Menon, V. P.; Otani, H.; Maulik, N. Statin and resveratrol in combination induces cardioprotection against myocardial infarction in hypercholesterolemic rat. *J. Mol. Cell. Cardiol.* **2007**, *42* (3), 508–516.

(6) Thirunavukkarasu, M.; Penumathsa, S. V.; Koneru, S.; Juhasz, B.; Zhan, L.; Otani, H.; Bagchi, D.; Das, D. K.; Maulik, N. Resveratrol alleviates cardiac dysfunction in streptozotocin-induced diabetes: Role of nitric oxide, thioredoxin, and heme oxygenase. *Free Radical Biol. Med.* **2007**, *43* (5), 720–729.

(7) Cui, J.; Juhasz, B.; Tosaki, A.; Maulik, N.; Das, D. K. Cardioprotection with grapes. *J. Cardiovasc. Pharmacol.* **2002**, *40* (5), 762–769.

(8) Baur, J. A.; Pearson, K. J.; Price, N. L.; Jamieson, H. A.; Lerin, C.; Kalra, A.; Prabhu, V. V.; Allard, J. S.; Lopez-Lluch, G.; Lewis, K.; Pistell, P. J.; Poosala, S.; Becker, K. G.; Boss, O.; Gwinn, D.; Wang, M.; Ramaswamy, S.; Fishbein, K. W.; Spencer, R. G.; Lakatta, E. G.; Le Couteur, D.; Shaw, R. J.; Navas, P.; Puigserver, P.; Ingram, D. K.; de Cabo, R.; Sinclair, D. A. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **2006**, *444* (7117), 337–342.

(9) Giovannini, C.; Straface, E.; Modesti, D.; Coni, E.; Cantafora, A.; De Vincenzi, M.; Malorni, W.; Masella, R. Tyrosol, the major olive oil biophenol, protects against oxidized-LDL-induced injury in Caco-2 cells. *J. Nutr.* **1999**, *129* (7), 1269–1277.

(10) Bertelli, A. A.; Migliori, M.; Panichi, V.; Longoni, B.; Origlia, N.; Ferretti, A.; Cuttano, M. G.; Giovannini, L. Oxidative stress and inflammatory reaction modulation by white wine. *Ann. N.Y. Acad. Sci.* **2002**, *957*, 295–301.

(11) Klatsky, A. L.; Friedman, G. D.; Armstrong, M. A.; Kipp, H. Wine, liquor, beer, and mortality. *Am. J. Epidemiol.* **2003**, *158* (6), 585–595.

(12) Waterhouse, A. L. Wine phenolics. *Ann. N.Y. Acad. Sci.* **2002**, *957*, 21–36.

(13) Cui, J.; Tosaki, A.; Cordis, G. A.; Bertelli, A. A.; Bertelli, A.; Maulik, N.; Das, D. K. Cardioprotective abilities of white wine. *Ann. N.Y. Acad. Sci.* **2002**, *957*, 308–316.

(14) Mansvelt, E. P.; van Velden, D. P.; Fourie, E.; Rossouw, M.; van Rensburg, S. J.; Smuts, C. M. The in vivo antithrombotic effect of wine consumption on human blood platelets and hemostatic factors. *Ann. N.Y. Acad. Sci.* **2002**, *957*, 329–332.

(15) Pellegrini, N.; Pareti, F. I.; Stabile, F.; Brusamolino, A.; Simonetti, P. Effects of moderate consumption of red wine on platelet aggregation and haemostatic variables in healthy volunteers. *Eur. J. Clin. Nutr.* **1996**, *50* (4), 209–213.

(16) Bertelli, A. A.; Migliori, M.; Panichi, V.; Origlia, N.; Filippi, C.; Das, D. K.; Giovannini, L. Resveratrol, a component of wine and grapes, in the prevention of kidney disease. *Ann. N.Y. Acad. Sci.* **2002**, *957*, 230–238.

(17) Murphy, E. Primary and secondary signaling pathways in early preconditioning that converge on the mitochondria to produce cardioprotection. *Circ. Res.* **2004**, *94* (1), 7–16.

(18) Brazil, D. P.; Hemmings, B. A. Ten years of protein kinase B signalling: a hard Akt to follow. *Trends Biochem. Sci.* **2001**, *26* (11), 657–664.

(19) Cardone, M. H.; Roy, N.; Stennicke, H. R.; Salvesen, G. S.; Franke, T. F.; Stanbridge, E.; Frisch, S.; Reed, J. C. Regulation of cell death protease caspase-9 by phosphorylation. *Science* **1998**, *282* (5392), 1318–1321.

(20) Webster, K. A. Aktion in the nucleus. *Circ. Res.* **2004**, *94* (7), 856–859.

(21) Jugdutt, B. I. Nitric oxide and cardioprotection during ischemia-reperfusion. *Heart Failure Rev.* **2002**, *7* (4), 391–405.

(22) Paxinou, E.; Weisse, M.; Chen, Q.; Souza, J. M.; Hertkorn, C.; Selak, M.; Daikhin, E.; Yudkoff, M.; Sowa, G.; Sessa, W. C.; Ischiropoulos, H. Dynamic regulation of metabolism and respiration by endogenously produced nitric oxide protects against oxidative stress. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98* (20), 11575–11580.

(23) Takano, H.; Manchikalapudi, S.; Tang, X. L.; Qiu, Y.; Rizvi, A.; Jadoon, A. K.; Zhang, Q.; Bolli, R. Nitric oxide synthase is the mediator of late preconditioning against myocardial infarction in conscious rabbits. *Circulation* **1998**, *98* (5), 441–449.

(24) Penumathsa, S. V.; Koneru, S.; Thirunavukkarasu, M.; Zhan, L.; Prasad, K.; Maulik, N. Secoisolariciresinol diglucoside: relevance to angiogenesis and cardioprotection against ischemia-reperfusion injury. *J. Pharmacol. Exp. Ther.* **2007**, *320* (2), 951–959.

(25) Koneru, S.; Penumathsa, S. V.; Thirunavukkarasu, M.; Vidavalur, R.; Zhan, L.; Singal, P. K.; Engelman, R. M.; Das, D. K.; Maulik, N. Sildenafil mediated neovascularization and protection against myocardial ischemia reperfusion injury in rats: probable role of Vegf/Angiopoietin-1. *J. Cell. Mol. Med.* In press, **2008**.

(26) Maulik, N.; Sato, M.; Price, B. D.; Das, D. K. An essential role of NFkappaB in tyrosine kinase signaling of p38 MAP kinase

regulation of myocardial adaptation to ischemia. *FEBS Lett.* **1998**, *429* (3), 365–369.
(27) Matsui, T.; Tao, J.; del Monte, F.; Lee, K. H.; Li, L.; Picard, M.; Force, T. L.; Franke, T. F.; Hajjar, R. J.; Rosenzweig, A. Akt activation preserves cardiac function and prevents injury after transient cardiac ischemia in vivo. *Circulation* **2001**, *104* (3), 330–335.
(28) Hausenloy, D. J.; Tsang, A.; Mocanu, M. M.; Yellon, D. M. Ischemic preconditioning protects by activating prosurvival kinases at reperfusion. *Am. J. Physiol.* **2005**, *288* (2), H971−976.
(29) Brunet, A.; Bonni, A.; Zigmond, M. J.; Lin, M. Z.; Juo, P.; Hu, L. S.; Anderson, M. J.; Arden, K. C.; Blenis, J.; Greenberg, M. E. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* **1999**, *96* (6), 857–868.
(30) Juhasz, B.; Thirunavukkarasu, M.; Pant, R.; Zhan, L.; Penumathsa, S.; Secor, E. R.; Srivastava, S.; Raychaudhuri, U.; Menon, V. P.; Otani, H.; Thrall, R. S.; Maulik, N. Bromelain induces cardioprotection against ischemia reperfusion injury through Akt/Foxo pathway in rat myocardium. *Am. J. Physiol.* In press, **2008**.
(31) Dimmeler, S.; Fleming, I.; Fisslthaler, B.; Hermann, C.; Busse, R.; Zeiher, A. M. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* **1999**, *399* (6736), 601–605.
(32) Gao, F.; Gao, E.; Yue, T. L.; Ohlstein, E. H.; Lopez, B. L.; Christopher, T. A.; Ma, X. L. Nitric oxide mediates the antiapoptotic effect of insulin in myocardial ischemia-reperfusion: the roles of PI3-kinase, Akt, and endothelial nitric oxide synthase phosphorylation. *Circulation* **2002**, *105* (12), 1497–1502.
(33) Du Toit, E. F.; Meiring, J.; Opie, L. H. Relation of cyclic nucleotide ratios to ischemic and reperfusion injury in nitric oxide-donor treated rat hearts. *J. Cardiovasc. Pharmacol.* **2001**, *38* (4), 529–538.
(34) Jones, S. P.; Bolli, R. The ubiquitous role of nitric oxide in cardioprotection. *J. Mol. Cell. Cardiol.* **2006**, *40* (1), 16–23.
(35) Hambrecht, R.; Adams, V.; Erbs, S.; Linke, A.; Krankel, N.; Shu, Y.; Baither, Y.; Gielen, S.; Thiele, H.; Gummert, J. F.; Mohr, F. W.; Schuler, G. Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase. *Circulation* **2003**, *107* (25), 3152–3158.
(36) Romashkova, J. A.; Makarov, S. S. NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling. *Nature* **1999**, *401* (6748), 86–90.
(37) Craig, R.; Wagner, M.; McCardle, T.; Craig, A. G.; Glembotski, C. C. The cytoprotective effects of the glycoprotein 130 receptor-coupled cytokine, cardiotrophin-1, require activation of NF-kappa B. *J. Biol. Chem.* **2001**, *27640*, 37621–37629.
(38) Peng, H. B.; Libby, P.; Liao, J. K. Induction and stabilization of I kappa B alpha by nitric oxide mediates inhibition of NF-kappa B. *J. Biol. Chem.* **1995**, *270* (23), 14214–14219.
(39) Matthews, J. R.; Botting, C. H.; Panico, M.; Morris, H. R.; Hay, R. T. Inhibition of NF-kappaB DNA binding by nitric oxide. *Nucleic Acids Res.* **1996**, *24* (12), 2236–2242.
(40) Grumbach, I. M.; Chen, W.; Mertens, S. A.; Harrison, D. G. A negative feedback mechanism involving nitric oxide and nuclear factor kappa-B modulates endothelial nitric oxide synthase transcription. *J. Mol. Cell. Cardiol.* **2005**, *39* (4), 595–603.
(41) Xuan, Y. T.; Tang, X. L.; Banerjee, S.; Takano, H.; Li, R. C.; Han, H.; Qiu, Y.; Li, J. J.; Bolli, R. Nuclear factor-kappaB plays an essential role in the late phase of ischemic preconditioning in conscious rabbits. *Circ. Res.* **1999**, *84* (9), 1095–1109.
(42) Mangi, A. A.; Noiseux, N.; Kong, D.; He, H.; Rezvani, M.; Ingwall, J. S.; Dzau, V. J. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat. Med.* **2003**, *9* (9), 1195–1201.
(43) Scherrer-Crosbie, M.; Ullrich, R.; Bloch, K. D.; Nakajima, H.; Nasseri, B.; Aretz, H. T.; Lindsey, M. L.; Vancon, A. C.; Huang, P. L.; Lee, R. T.; Zapol, W. M.; Picard, M. H. Endothelial nitric oxide synthase limits left ventricular remodeling after myocardial infarction in mice. *Circulation* **2001**, *104* (11), 1286–1291.
(44) Jones, S. P.; Greer, J. J.; van Haperen, R.; Duncker, D. J.; de Crom, R.; Lefer, D. J. Endothelial nitric oxide synthase overexpression attenuates congestive heart failure in mice. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100* (8), 4891–4896.
(45) Chen, L. L.; Yin, H.; Huang, J. Inhibition of TGF-beta1 signaling by eNOS gene transfer improves ventricular remodeling after myocardial infarction through angiogenesis and reduction of apoptosis. *Cardiovasc. Pathol.* **2007**, *16* (4), 221–230.

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