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Cardioprotective effect of resveratrol via HO-1 expression involves p38 map kinase and PI-3-kinase signaling, but does not involve NFκB

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Abstract

Recent studies have demonstrated that resveratrol (trans-3,4′,5-trihydroxy stilbene), a phytoalexin found in the skin and seeds of grapes, can pharmacologically precondition (PC) the heart through a nitric oxide (NO)-dependent and adenosine receptors-mediated mechanism. Since NO can induce the expression of heme oxygenase-1 (HO-1), we examined if HO-1 induction has a direct role in resveratrol-preconditioning of the heart. Eight groups of rats were studied during 7 days: (i) control rats; (ii) rats receiving resveratrol (gavage, 2.5 mg/kg); (iii) rats injected tin protoporphyrin (SnPP), a HO-1 inhibitor, i.p. on days 1, 3 and 6; (iv) rats injected 202190 (SB), a p38MAPK inhibitor, i.p. for 7days; (v) rats injected 294002 (LY), a Akt inhibitor, i.p. for 7days; (vi) rats receiving resveratrol and SnPP; (vii) rats receiving resveratrol and SB; and (viii) rats receiving resveratrol and LY. After the treatments, the rats were sacrificed, and the hearts isolated and subjected to 30 min global ischemia followed by 2 h of reperfusion. The results shown a significant cardioprotection with resveratrol as evidenced by superior post-ischemic ventricular recovery, reduced myocardial infarct size, and decreased number of apoptotic cardiomyocytes. SnPP treatment abolished the cardioprotective effect of resveratrol. Resveratrol induced the activation of nuclear factor kappa-β(NFκB), the phosphorylation of p38MAP kinase β and Akt, as well as the inhibition of p38 MAP kinase α; all these effects but the activation of NFκB, were completely reversed by treatment with SnPP. These results indicate that resveratrol generates cardioprotection by preconditioning the heart by HO-1-mediated mechanisms, which are regulated by p38MAP kinase and Akt survival signaling, but non-dependent on NFκB activation.

Keywords: *Resveratrol, heme oxygenase, p38 MAP kinase, Akt, NFκB*

Introduction

Resveratrol is a phenolic phytoalexin present in grape skins and wines, especially red wines [1,2]. Resveratrol exerts a wide variety of biological actions including estrogenic [3], anti-platelet and anti-inflammatory activities [6,7], as well as protection of low-density lipoprotein (LDL) from oxidation [4,5]. Resveratrol has also been found to protect kidney, brain and heart from ischemia/reperfusion injury [8–10].

Substantial evidence indicates that resveratrol pharmacologically preconditions (PC) heart to resist ischemia–reperfusion insults. Both, nitric oxide (NO)-dependent pathways [11,12] and the activation of adenosine receptors [13], are mechanisms that could be mediating the heart preconditioning by resveratrol. In cardiomyocytes, the NO-mediated regulation of cardioprotective enzymes by NO-mediated mechanisms is crucial for cell survival.

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Heme oxygenases (HO) are the most important enzymes for heme degradation. The three isoforms of the enzyme are HO-1, -2 and -3. The inducible form, HO-1, is commonly found in the heart and it has been found to display relevant cardioprotective actions by CO-mediated mechanisms [14]. Ancilliary, the activity of HO leads to the formation of billiverdin and bilirubin [20], antioxidant compounds with potential protective actions

Various studies have revealed that the induction of HO-1 expression, secondary to oxidative stress, hypoxia, ischemia/reperfusion, and different environmental factors [15–19], resulted in CO-mediated cardioprotection. In HO-1 knockout mice model, HO-1 mediates the formation of endogenous CO, putative cellular messenger, production attenuating ischemia/reperfusion-induced ventricular fibrillation [21]. In rats heart also the protective effect of CO through the HO-1 activation is observed [21]. In another study, in rabbit model, it has been shown HO-1 plays an important role in attenuating post-ischemic myocardial injury through the activation of HIF-1 [22]. Recently, in both rats and mice model it has been shown that in diabetes, HO-1 significantly decrease the infarct zone in the myocardium caused by ischemia/reperfusion [23,24]. Numerous other studies also confirm the involvement of HO-1 in cardiovascular health both *in vivo* and *in vitro* [25–29].

In view of the fact that resveratrol can pharmacologically PC the heart [30–35], NO is both involved in preconditioning [36–38] and in the regulation of HO-1 expression [39,40], and resveratrol can induce the expression of HO-1 [37–39], we sought to examine potential mechanisms for resveratrol protection of the heart associated to HO-1 induction. We characterized the activation of NFκB and MAP kinases associated to the induction of HO-1 by resveratrol in a model of heart ischemia–reperfusion. The results of our study showed that resveratrol preconditioned the heart by activating p38MAPKβ-Akt signaling, but independently of NFκB activation.

Materials and methods

Resveratrol

Resveratrol (trans-3,4′,5-trihydroxystilbene), a natural phytoalexin and SB 202190 were obtained from Sigma Chemical Co. (St Louis, MO, USA). A highly specific inhibitor of HO-1 activity, tin-protoporphyrin IX (SnPP), was purchased from Porphyrin Products (Logan, Utah). LY 294002 was purchased from Calbiochem (San Diego, CA, USA). Resveratrol was dissolved in DMSO, aliquoted, and kept at 4°C. SnPP was dissolve in alkaline (saturated $NaHCO_3$) aqueous solution, aliquoted, and frozen at −20°C with light protective cover. Antibodies for HO-1, p38α, p38β and Akt were obtained from Cell Signaling Technologies, (Danvers, MA, USA).

Treatment of rats with resveratrol and resveratrol plus SnPP

Sprague–Dawley male rats (250–275 g) obtained from Harlan (Indianapolis, IN, USA), were gavaged with 0.5 ml of 50% (V/V) ethanol (control group) or with resveratrol (2.5 mg/kg of body weight dissolved in 50% (V/V) ethanol) (resveratrol group) using a stomach needle (1.2 mm diameter) every day for 7 days. A third set of rats, gavaged with vehicle, was injected with SnPP (50 μmol/kg body weight, i.p.), on days 1, 3 and 6 (SnPP group). Another group of rats (resveratrol-SnPP) was gavaged with resveratrol, and follow the same schedule of SnPP treatment than the SnPP group. All rats used in this study received humane care in compliance 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 (Pub. No. NIH85-23, revised 1985).

Treatment of rats with SB 202190 and LY 294002 with or without resveratrol

Sprague–Dawley male rats (250–275 g) were injected with 20 μM of SB 202190 and 3 μM of LY 294002 regularly for 7 days, i.p. (SB and LY groups) The seventh and eighth groups the same dose of resveratrol was gavaged with regular i.p. injection of SB (resveratrol-SB group) and LY (resveratrol-LY group), respectively.

Isolated working heart preparation

After 7 days of treatment, all groups of rats were anesthetized by i.p. injection of 80 mg/kg body weight sodium pentobarbital (Abbott; North Chicago, IL, USA). After intravenous administration of heparin (Elkins-Sinn; Cherry Hill, NJ, USA), at a dose of 500 IU/kg of body weight, the chests were opened, and the heart from each rat was rapidly excised and mounted on a non-recirculating Langendorff perfusion apparatus [7]. The perfusion buffer used in this study consisted of a modified Krebs–Henseleit bicarbonate buffer (KHB) (in mM: 118 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 25 NaHCO3, 10 glucose and 1.7 CaCl2), pH 7.4, gassed with 95% O2–5% CO2, and filtered through a 5 μm filter. The KHB was maintained at 37°C and gassed continuously for the entire duration of the experiment. Left atrial cannulation was then carried out, and, after stabilization for 10 min in the retrograde perfusion mode, the circuit was switched to the antegrade

working mode, which allowed for the measurement of myocardial contractility as well as aortic and coronary flow (CF). Essentially, this is a left heart preparation in which the heart is perfused with a constant preload of 17 cm H_2O (being maintained by means of a Masterflex variable speed modular pump, Cole Parmer Instrument; Vernon Hills, IL, USA) and pumps against an afterload of 100 cm H_2O. At the end of 10 min, after the attainment of steady-state cardiac function, baseline functional parameters were recorded. The circuit was then switched back to the retrograde mode, and the hearts subjected to 30 min of global ischemia followed by 120 min of reperfusion with the same KHB buffer. The first 10 min of reperfusion was in the retrograde mode to allow for post-ischemic stabilization and thereafter in the antegrade-working mode to allow for assessment of functional parameters, which were recorded at 10, 30, 60 and 120 min, into reperfusion.

Cardiac function assessment

Aortic pressure was measured using a Gould P23XL pressure transducer (Gould Instrument Systems Inc., Valley View, OH, USA) connected to a side arm of the aortic cannula, the signal was amplified using a Gould 6600 series signal conditioner and monitored on a CORDAT II real-time data acquisition and analysis system (Triton Technologies, San Diego, CA, USA) [7]. Heart rate (HR), left ventricular developed pressure (LVDP) (defined as the difference of the maximum systolic and diastolic aortic pressures), and the first derivative of developed pressure ($LVdP/dt$) were all derived or calculated from the continuously obtained pressure signal. Aortic flow (AF) was measured using a calibrated flow meter (Gilmont Instrument Inc., Barrington, IL, USA) and CF was measured by timed collection of the coronary effluent dripping from the heart.

Infarct size estimation

At the end of reperfusion, a 10% (w/v) solution of triphenyl tetrazolium in phosphate buffer was infused into aortic cannula for 20 min at 37°C [13]. The hearts were excised and stored at −70°C. Sections (0.8 mm) of frozen heart were fixed in 2% para-formaldehyde, placed between two cover slips and digitally imaged using a Microtek Scan Maker at 600z. To quantitate the areas of interest in pixels, a NIH image 5.1 (a public domain software package) was used. The infarct size was quantified and expressed in pixels.

Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay for assessment of apoptotic cell death

Immunohistochemical detection of apoptotic cells was carried out using TUNEL [13] with APOP TAG kit (Oncor, Gaithersburg, MD, USA). The heart tissues were immediately put in 10% formalin and fixed in an automatic tissue-fixing machine. The tissues were carefully embedded in the molten paraffin in metallic blocks, covered with flexible plastic molds, and kept under freezing plates to allow the paraffin to solidify. The metallic containers were removed, and tissues became embedded in paraffin on the plastic molds. Before analyzing tissues for apoptosis, tissue sections were deparaffinized with xylene and washed in succession with different concentrations of ethanol (95, 70 and 100%). Then, tissues were incubated again with mouse monoclonal antibody recognizing cardiac myosin heavy chain to specifically recognize apoptotic cardiomyocytes. The fluorescence staining was viewed with a confocal laser microscope. The number of apoptotic cells was counted and expressed as a percentage of total myocyte population.

Western blot analysis

Left ventricles from the hearts were homogenized in a buffer containing 25 mM Tris–HCl, 25 mM NaCl, 1 mM orthovanadate, 10 mM NaF, 10 mM pyrophosphate, 10 mM okadaic acid, 0.5 mM EDTA, and 1 mM phenyl methyl sulfonyl fluoride [7]. Protein (100 μg) of each heart homogenate was incubated with 1 μg of antibody against p38α, p38β, phospho-specific p38α and phospho-specific p38β, Akt, and phospho-Akt for 1 h at 4°C. The immune complexes were precipitated with protein A–sepharose; immunoprecipitates were separated by SDS-polyacrylamide gel electrophoresis, and immobilized on polyvinylidene difluoride membrane. The membrane was immunoblotted with PY20 to evaluate the phosphorylation of the compounds. The membrane was then stripped and reblotted with specific antibodies against glucose-6-phosphate dehydrogenase, which served as loading control. The resulting blots were digitized, subjected to densitometric scanning using a standard NIH Image program, and normalized against loading control.

NFκB analysis

To determine DNA binding of NFκB, nuclear proteins were isolated from the heart as described previously [39]. In short, about 150 mg of left ventricle from the heart tissue was homogenized in ice-cold Tris-buffered saline (TBS) and centrifuged at 3000*g* for 5 min at 4°C. The pellet was resuspended by gentle pipetting in 1.5 ml of ice-cold hypotonic buffer containing 10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF and 1 μM each of aprotinin, pepstatin, and leupeptin. The solution was allowed to swell on ice for 15 min. After addition of 100 mM of 10% Nonidet P-40, the tube was vigorously vortexed for 45 s. This

homogenate was centrifuged for 30 s at 4°C in a microcentrifuge tube. The supernatant contained the cytoplasmic protein. The nuclear pellet was resuspended in a solution containing 20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF and 1 μM each of aprotinin, pepstatin and leupeptin. The tubes were vigorously shaken at 4°C for 30 min on a shaking platform. The extracts were then centrifuged and the supernatants were stored at −70°C. Protein concentration was estimated using the Pierce Protein Assay kit (Pierce Chemical Co, Rockford, IL, USA).

NFκB oligonucleotide (AGTTGAGG-GGACTT-TCCCAGG) (2.5 μl of 20 nmol/μl) was labeled using T4 polynucleotide kinase as previously described. The binding reaction mixture contained in a total volume of 20.2 μl, 0.2 μl DTT (0.2 M), 1 μl BSA (20 mg/ml), 4 μl poly(dI–dC) (0.5 μg/μl), 0.2 μl buffer D, 4 μl buffer *f*, 2 mM 32P-oligo (0.5 ng/μl) and 7 μl extract containing 10 μg protein. The composition of buffer D + was 20 mM HEPES, pH 7.9, 20% glycerol, 100 mM KCl, 0.5 mM EDTA, 0.25% Nonidet P-40 while buffer F contained 20% Ficoll 400, 100 mM HEPES, pH 7.9, and 300 mM KCl. Incubation was carried out for 20 min at room temperature. Until the dye hit the bottom 10 ml of the solution was loaded onto a 4% acrylamide gel and separated at 80 V. After electrophoresis, gels were dried and exposed to Kodak X-ray film at −70°C. Autoradiographic results were evaluated quantitatively by an image analyzer. The binding signal from each sample was measured in the same sized area. The non-specific binding was determined by adding at least 60-fold excess of unlabelled DNA probes to the assay mixture. Therefore, the specific binding was calculated by subtracting the non-specific binding from total binding.

Statistical analysis

The values for myocardial functional parameters, total and infarct volumes and infarct sizes, and cardiomyocyte apoptosis are all expressed as the mean ± SEM. Analysis of variance test followed by Bonferroni's correction was first carried out to test for differences between the mean values of all groups. If differences were established, the values of the treated groups were compared with those of the control group by a modified *t*-test. The results were considered significant if $p < 0.05$.

Results

Cardioprotective effect of resveratrol

There were no differences in baseline cardiac function between the four groups, control SnPP, resveratrol, and resveratrol-SnPP (Figures 1 and 2).

There were no significant differences among the four groups in terms of HR and CF. HR at baseline was 312 ± 31, 435 ± 16, 427 ± 15 and 410 ± 12 beat/min, for control, resveratrol, SnPP and resveratrol-SnPP groups (results not shown). CF was 29.8 ± 0.9, 21.4 ± 2.1, 22.4 ± 2.2 and 23.6 ± 2.1 ml/min, for control, resveratrol, SnPP and resveratrol-SnPP groups, respectively. Both, HR and CF did not change significantly after IR.

On reperfusion, the values of all functional parameters evaluated, i.e. LVDP, d(LVP)/d*t*, and AF, decreased as function of time in the four groups as compared with the respective baseline values. LVDP decreased from 127 ± 3 mm Hg (baseline) to 43 ± 8 mm Hg after 120R; LV dp/dt decreased from 3118 ± 115 mm Hg/s (baseline) to 890 ± 87 mm Hg/s after 120R; and AF decreased from 71.6 ± 5.2 ml/min (baseline) to 4.3 ± 1.4 ml/min after 120R.

In accordance with our previous study, resveratrol-treated hearts displayed a better maintenance of myocardial function post-ischemia. The cardio protective effects of resveratrol were evidenced by the significant prevention in LVDP, LV dp/dt, and AF from R-30 onward (Figure 1). LVDP in the resveratrol group was significantly higher ($p < 0.05$) than in the control group at R-60 (113 ± 1 vs. 88 ± 10 mm Hg) and at R-120 (95 ± 2 vs. 43 ± 8 mm Hg). In the resveratrol group d(LVP)/d*t* was significantly higher ($p < 0.05$) than in the control group at R-30 (3302 ± 122 vs. 2472 ± 235 mm Hg/s), at R-60 (3000 ± 62 vs. 1881 ± 403 mm Hg/s) and at R-120 (2096 ± 125 vs. 890 ± 87 mm Hg/s). AF was markedly higher ($p < 0.05$) in the resveratrol group than in the control group at R-30 (62.6 ± 2.4 vs. 36.0 ± 13.0 ml/min); at R-60 (47.2 ± 3.8 vs.19.2 ± 6.5 ml/min); and at R-120 (10.5 ± 1.7 vs. 4.3 ± 1.4 ml/min).

No relevant differences in the values of LVDP, LV dp/dt, and blood flow were observed between the control and the SnPP and the resveratrol–SnPP group, indicating in the resveratrol–SnPP group, the cardioprotective changes observed in the resveratrol group were lost or drastically diminished.

Both with SB and LY injection there is no significant difference in any of the functional parameters compare to the control group (data not shown). But when these inhibitors were provided together with resveratrol, the cardio protective effect of resveratrol was abolished. The significant decrease in LVDP from 60 min reperfusion (Figure 1) and onwards and also the significant decrease in LV dp/dt from 30 min onwards (Figure 1) as well as the significant decrease in AF from 30 min reperfusion (Figure 2) with SB and LY both used along with resveratrol compare to resveratrol gavaged group, confirmed that the cardioprotective effect of resveratrol was abolished by these two inhibitors.

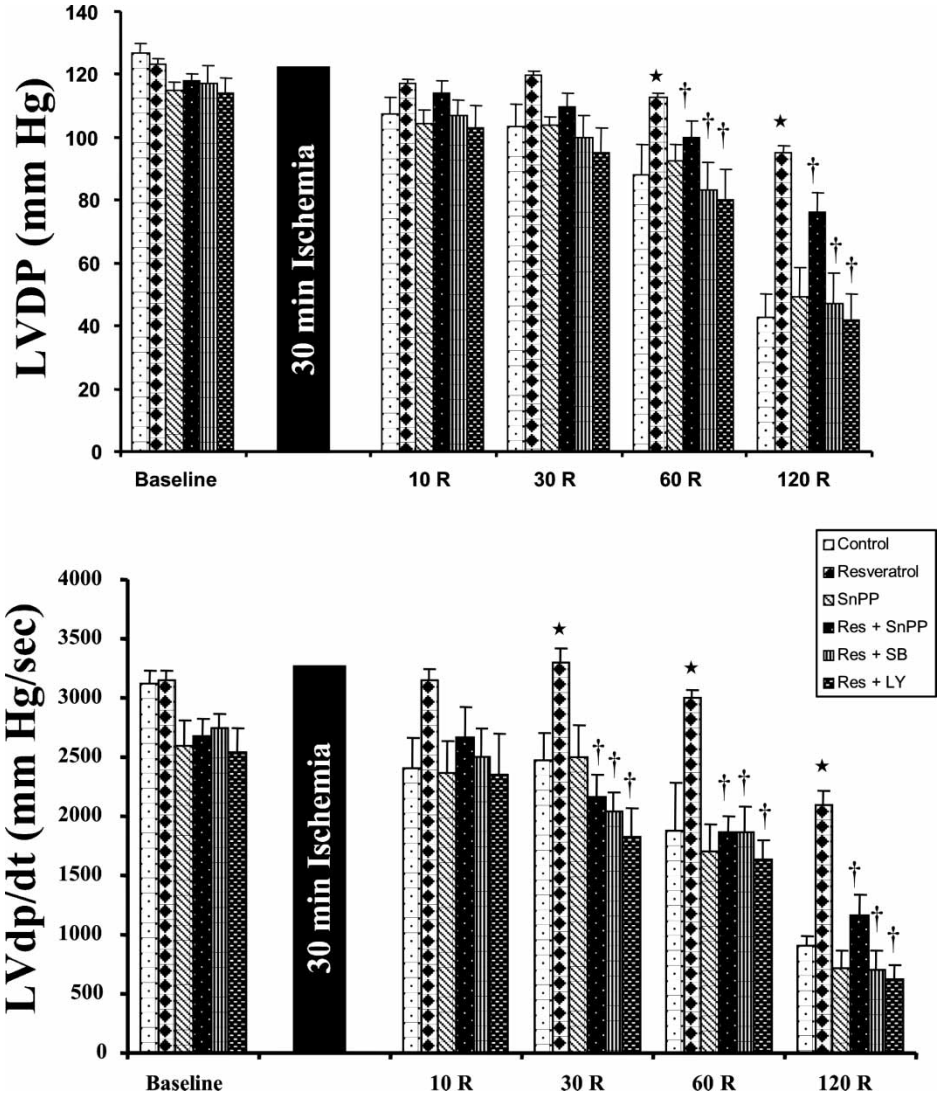

Figure 1. The effects of resveratrol and its inhibitor on LVDP (top) and LV dp/dt (bottom). Results are shown for $n = 6$ per group. $\star p < 0.05$ vs. control; $^{\dagger}p < 0.05$ vs. resveratrol.

Effects of resveratrol on myocardial infarct size

Infarct size (infarct vs. total area at risk) determined after the IR period was significantly reduced in the resveratrol group (21%) as compared to the control (34%), SnPP (35%), resveratrol–SnPP groups (33%), resveratrol–SB group (31%) and resveratrol–LY group (35%) (Figure 3A). Only SB or LY treatment did not change the infarct size significantly compare to control (data not shown).

Effects of resveratrol on cardiomyocyte apoptosis

The percent of apoptotic cardiomyocytes was significantly reduced in the resveratrol group (6%) as compared to the control (22%), SnPP (23%), resveratrol–SnPP groups (24%), resveratrol–SB group (18%) and resveratrol–LY group (25%) (Figure 3B). Only SB or LY treatment did not change the apoptotic cell death significantly compare to control (data not shown).

Effects of resveratrol on HO-1 induction and NFκB–DNA binding activity

As shown in Figure 3, resveratrol caused a 10-fold induction of HO-1. The activity of HO-1 was completely inhibited in the SnPP and resveratrol-SnPP groups. Resveratrol treatment significantly increased the NFκB–DNA binding activity (Figure 4) as compared to both, control and SnPP groups.

Effects of resveratrol on the expression of p38 MAPKs, Akt and their phosphorylated products

Compared to the control group, the treatment with resveratrol induced the expression of p38β and phospho p38β and enhanced the phosphorylation of

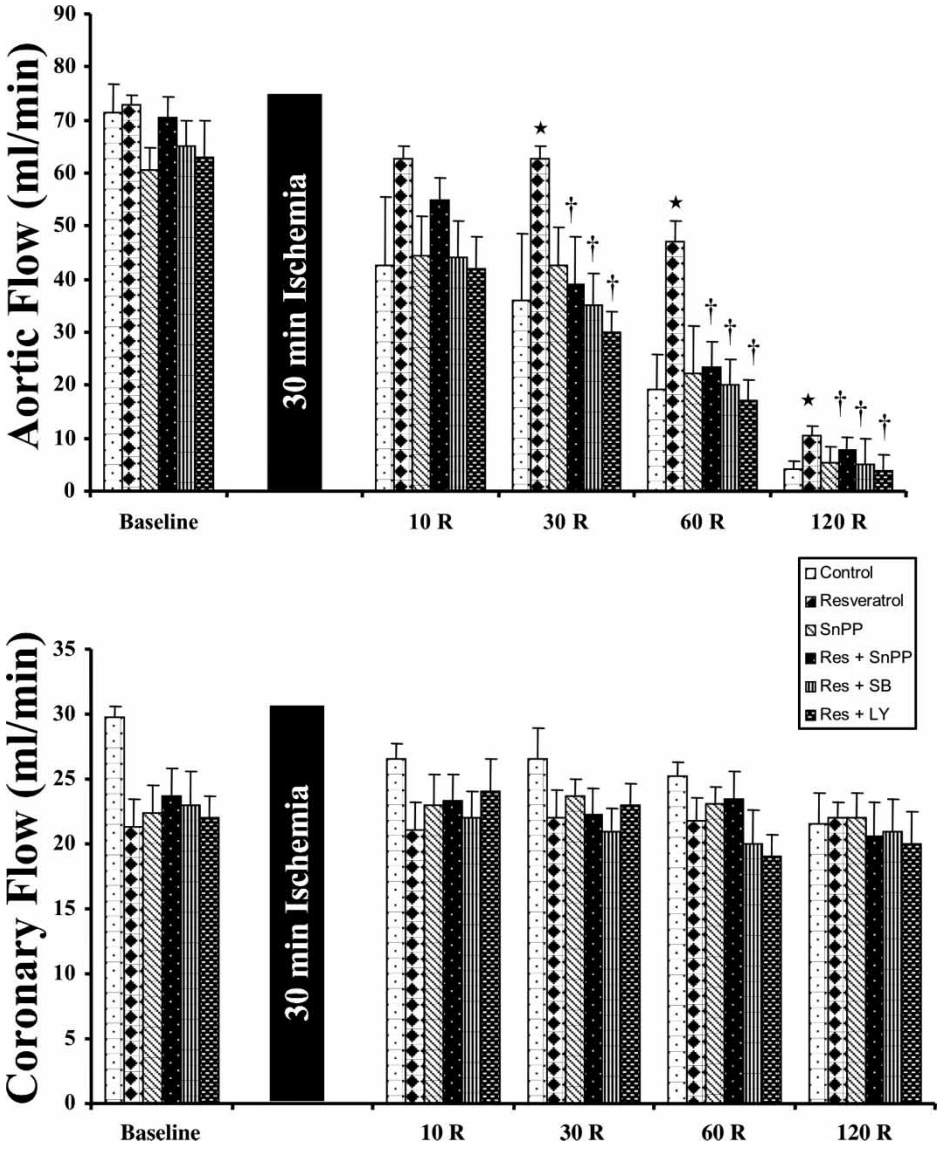

Figure 2. The effects of resveratrol and its inhibitor on AF (top) and CF (bottom). Results are shown for $n = 6$ per group. $^{\star}p < 0.05$ vs. control; $^{\dagger}p < 0.05$ vs. resveratrol.

Akt 12-, 17- and 11-fold, respectively. SnPP treatment completely abolished the activation of p38β and Akt (Figure 4). In contrast, resveratrol reduced the expression of p38α significantly, which was reversed by SnPP (Figure 4).

Discussion

The results of the present study demonstrated that short term resveratrol consumption preconditioned the rat heart as evidenced by the improvement of post-ischemic ventricular recovery, the reduced myocardial infarct size, and the decreased number of apoptotic cardiomyocytes observed after ischemia reperfusion. The administration of SnPP, a highly specific HO-1 inhibitor, abrogated several of the cardioprotective effects of resveratrol, suggesting a role of HO-1 in resveratrol-mediated cardioprotection. Interestingly, the resveratrol-treatment enhanced the NFκB–DNA binding activity; and such activation of NFκB was not affected by the SnPP treatment. In addition, resveratrol activated, p38β and its phosphorylation, while reduced the induction of p38α and its phosphorylation. Resveratrol also enhanced the phosphorylation of Akt. These results suggest that HO-1 activation and the p38β-Akt survival signals, but not NFκB activation, are a necessary condition for resveratrol mediated cardioprotection.

Ischemia–reperfusion generate both, functional (blood pressure and blood flow) and histological (necrotic heart tissue and apoptotic cells) changes that were prevented by resveratrol. SnPP-treatment reversed the cardioprotection displayed by resveratrol, supporting the participation of HO-1. The association between IR damage and ROS, backs up the antioxidant action of HO-1 in the cardioprotection.

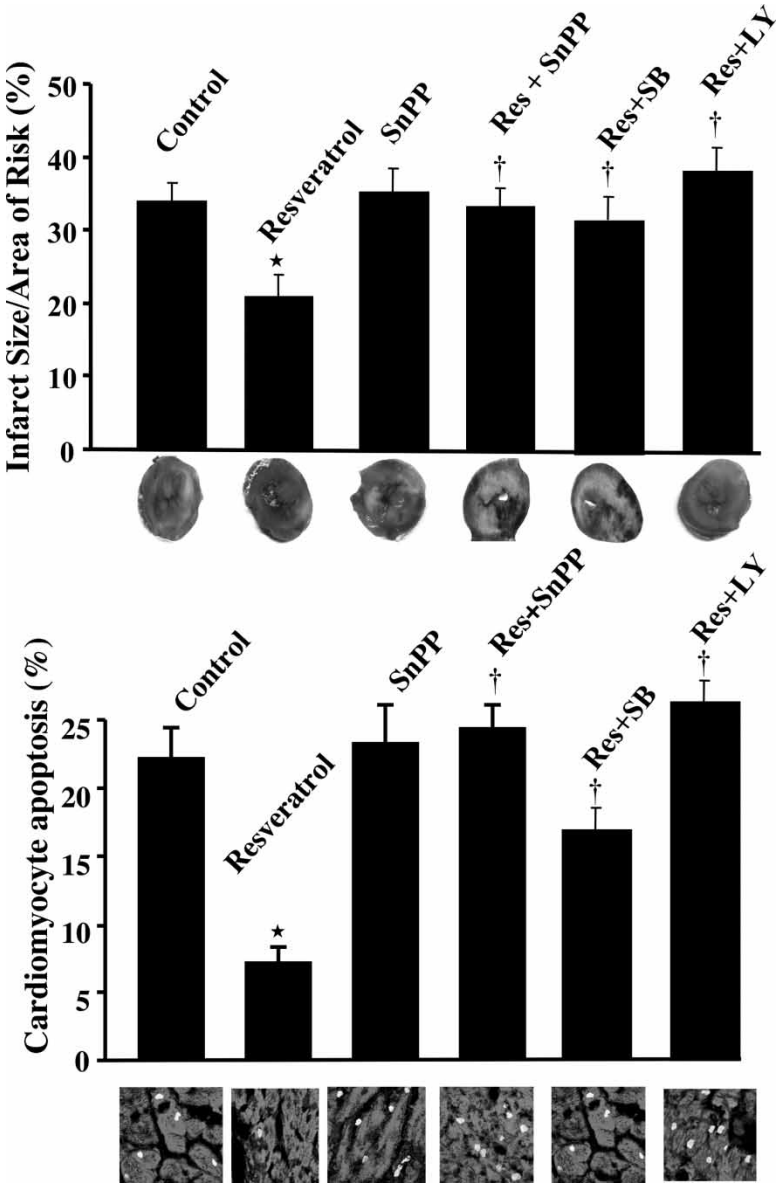

Figure 3. The effects of resveratrol and its inhibitor on infarct size (top) and cardiomyocyte apoptosis (bottom). Results are shown for $n = 6$ per group. $\star p < 0.05$ vs. control; $^{\dagger}p < 0.05$ vs. resveratrol.

The cardioprotective ability of resveratrol mediated by HO-1 is further supported by evidence that in different tissues resveratrol induces HO-1 and protect from damage. Resveratrol induced HO-1 in the neuronal culture cells [41]; in PC12 cells, resveratrol upregulated HO-1 expression, via activation of NF-E2-related factor 2 [44]; the cytoprotective action of resveratrol in astrocytes was related to an increase of HO-1 mRNA, and reversed by treatment with zinc protoporphyrin IX, a HO-1 inhibitor [42]; resveratrol stimulation of HO-1 was attributed to its ability to provide cellular resistance against oxidative stress in brain [45]; a concentration-dependent induction of HO-1 by resveratrol was observed in human aortic smooth muscle cells [43]. Very recently, a natural analog of resveratrol, piceatannol, was found to upregulate HO-1 expression via protein kinase C and tyrosine kinase pathways [46].

Although HO-1 can be implicated in the cardioprotective ability of resveratrol, the mechanism of action remains to be elucidated. To fill up this gap, we examined the contribution of NFκB, p38 MAPK and Akt as essential signaling molecules that could be involved in the activation of HO-1 by resveratrol.

NFκB activation was found to play an essential role in preconditioning, and its downregulation abolishes the cardioprotective abilities of preconditioning [47]. In the present study, we confirmed that resveratrol significantly increased the NFκB–DNA binding activity. However, the fact that NFκB remains

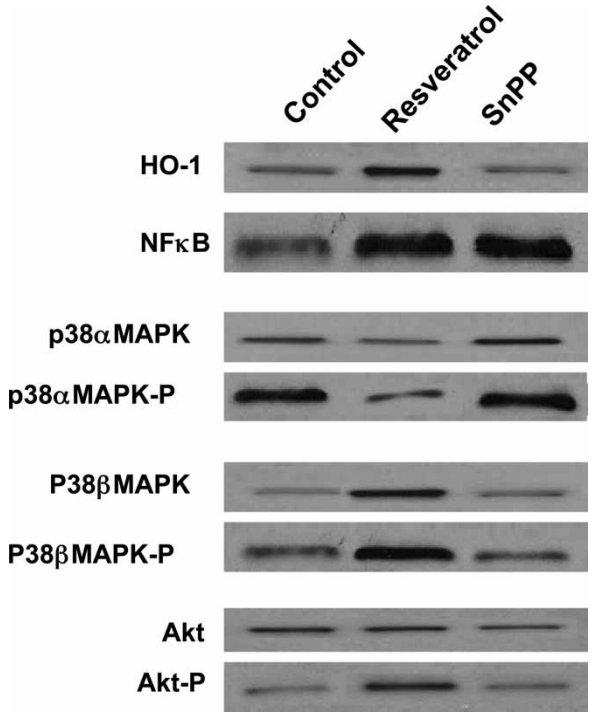

Figure 4. The effects of resveratrol and its inhibitor on the induction of the expression of HO-1, NFκB, p38MAPKα, p38MAPKβ and Akt and their phosphorylated products.

activated when the HO-1 expression was blocked with SnPP, suggests that: (i) HO-1 induction and NFκB activation are parallel events, and HO-1 may not exert its protective effects through NFκB; or (ii) HO-1 induction occurs downstream of NFκB activation and hence HO-1 inhibition does not modify the DNA binding ability of NFκB.

Numerous studies exist in the literature demonstrating a crucial role of p38MAPK in both ischemic [47–49] and pharmacological preconditioning by resveratrol [7,10–13]. Out of the four isomers of p38MAPK, α and β seems to play a regulatory role in preconditioning, p38α being detrimental and p38β being protective. In the present study, resveratrol, significantly activated p38β while inhibiting p38α, indicating that resveratrol eliminated the death signal while activating the survival signal. Interestingly, the changes in the α and β p38MAPKs by resveratrol were completely reversed by SnPP, supporting the relevance of the HO-1 dictating the regulation of MAPKs favoring cell survival.

Under the same concept, it is accepted that the inhibition of PI-3 kinase and/or Akt phosphorylation abolishes the cardioprotection [7,13]. Our observations that resveratrol increased the phosphorylation of Akt, and that SnPP blocked such activation, further support the participation of HO-1 in the phsophorylation of key molecules that determine intracellular signals that will define cell survival.

How HO-1 is regulating cell survival can not be drawn from the present results. However, it can be hypothesized that by providing antioxidant protection, HO-1 is generating a cell reducing capacity that is necessary to control the ischemia–reperfusion-mediated oxidant generation. A high oxidant production can lead to the lost of cell phosphorylation capacity which is necessary to prevent the apoptosis.

Finally, it is important to put the present results in the context of diet and health. Nevertheless the ischemia–reperfusion model can be of limited relevance for the diet, it is a model of oxidative stress, and it is well known the involvement of oxidative stress in the development of a number of diseases. The antioxidant actions of resveratrol are well supported by its chemical structure, but the tissue levels that can be reached after the consumption of a resveratrol-rich food, i.e. wine, are incompatible with a physiological radical scavenging action. Then, the possibility that resveratrol can regulate cell signaling events, which can be achieve at significantly lower concentration, supports dietary and pharmacological effects of resveratrol.

In conclusion, the results of our study demonstrated for the first time that resveratrol, at least in part, mediates cardioprotection by activating HO-1. HO-1 in turn generates a survival signal by upregulating p38β while downregulating p38α simultaneously increasing the phosphorylation of Akt.

Acknowledgements

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