Free Radical Biology & Medicine 46 (2009) 573–578

Contents lists available at ScienceDirect

Free Radical Biology & Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Expression of the longevity proteins by both red and white wines and their cardioprotective components, resveratrol, tyrosol, and hydroxytyrosol

Subhendu Mukherjee [a], Istvan Lekli [a], Narasimman Gurusamy [a], Alberto A.A. Bertelli [b], Dipak K. Das [a,*]

[a] Cardiovascular Research Center, University of Connecticut School of Medicine, Farmington, CT 06030-1110, USA
[b] Department of Human Anatomy, University of Milan, Milan, Italy

ARTICLE INFO

Article history:
Received 2 September 2008
Revised 15 October 2008
Accepted 7 November 2008
Available online 24 November 2008

Keywords:
White wine
Red wine
Resveratrol
Tyrosol
Hydroxytyrosol
PBEF
SirT1
FoxO1
Longevity
Cardioprotection

ABSTRACT

Resveratrol increases longevity through SirT1, which is activated with NAD^+ supplied by an anti-aging enzyme PBEF. SirT1 interacts with an anti-aging transcription factor, FoxO1, which is negatively regulated by Akt. Since white wine could have similar health benefits as red wine, we determined if white wine and its cardioprotective components possess anti-aging properties by feeding rats with these compounds. The hearts expressed SirT, FoxO, and PBEF in the order of white wine>resveratrol>tyrosol>hydroxytyrosol>red wine, while cardioprotection shown by reduction of infarct size and cardiomyocyte apoptosis followed a different pattern: resveratrol>red wine>hydroxytyrosol>white wine>tyrosol, suggesting the existence of different signaling mechanisms for the induction of longevity and survival.

Published by Elsevier Inc.

Introduction

A significant number of reports exist in the literature indicating that resveratrol, a natural phytoalexin found in grape skins and other plant materials as well as in red wine, can activate the longevity assurance genes Sirtuins (SirTs) [1–5]. Resveratrol was shown to extend the life span in Drosophila [6] and *Caenorhabditis elegans* [7] as well as in vertebrates such as short-lived fish Northobranchius [8]. Although no direct experimental evidence exists, red wine has been assumed to prolong the life span because of the presence of resveratrol in red wine.

Pre-B cell colony-enhancing factor (PBEF), also known as visfatin, is a highly conserved 52-kDa protein that regulates cellular levels of NAD^+ as well as NAD-dependent enzymes such as SirTs [9]. NAD^+ is an essential cofactor for NAD-dependent histone deacetylases (HDACs), SirTs. There are seven members in the SirT family (SirT 1–7), which differ with respect to their subcellular localizations [10]. PBEF was found to extend the life span of human smooth muscle cells by activating SirT1, which could deacetylate and suppress the function of FoxO [11]. FoxO comprises three functionally related proteins, FoxO1, FoxO3a, and FoxO4, all of which are known to interact with SirTs [12]. PI-3-kinase-Akt survival signaling pathway negatively regulates FoxO by phosphorylation of FoxO [13]. Thus phosphorylated FoxO becomes inactivated and comes out of the nucleus leading to ubiquitination followed by proteasomal degradation [14].

Most of the health benefits of red wine are related to cardioprotection [15]. Recent studies have indicated that some white wines could have similar health benefits as red wine. For example, a selected group of white wines from Italy and Germany could reduce oxidative stress and inflammatory response [16]. Another recent study using a French wine showed a similar degree of protection against early atherosclerosis compared to that produced by sparkling red wine [17]. A related study demonstrated an anti-inflammatory action of white wine consumption [18]. A study from our own laboratory also demonstrated that some white wines could reduce myocardial ischemic injury to the same extent as that of red wine [19]. Another more recent study from our laboratory showed that tyrosol and/or hydroxytyrosol that are present in white wine may be responsible for cardioprotection [20]. Nevertheless, no information is available if similar to resveratrol and red wine, tyrosol/hydroxytyrosol and white wine have any role in the prolongation of life span. To fill this gap, we

Abbreviations: I/R, ischemia–reperfusion; HDAC, histone deacetylase; GAPDH, glyceraldehyde-6-phosphate dehydrogenase; PBEF, pre-B cell colony-enhancing factor; SirT, Sirtuin; TUNEL, terminal deoxynucleotidyl transferase nick end labeling.

* Corresponding author. Fax: +1 860 679 4606.
E-mail address: DDAS@NEURON.UCHC.EDU (D.K. Das).

0891-5849/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.freeradbiomed.2008.11.005

undertook a study comparing the ability for red wine vs white wine and their cardioprotective components for the expression of longevity proteins. The results demonstrated that the hearts expressed SirTs, FoxOs, and PBEF in the order of white wine>resveratrol>tyrosol>hydroxytyrosol>red wine while cardioprotection shown by reduction of infarct size and cardiomyocyte apoptosis followed a different pattern: resveratrol>red wine>hydroxytyrosol>white wine>tyrosol. Also, we recently showed that phosphorylation of Akt followed the same pattern-like cardioprotection [20], suggesting the existence of different signaling mechanisms for the induction of longevity proteins and survival signal.

Materials and methods

Animals

All animals received humane care in compliance with the principles of the laboratory animal care formulated by the National Institutes of Health (Publication Number NIH 85-23, revised 1996). Sprague-Dawley rats weighing 250–300 g were gavaged for 14 days with experimental compounds, and then the animals were sacrificed for isolated working heart preparation. The rats were randomly assigned to one of the following groups: (i) control (water only); (ii) alcohol control (1 ml 12%); (iii) white wine (6.5 ml/kg); (iv) red wine (6.5 ml/kg); (v) resveratrol (2.5 mg/kg); (vi) tyrosol (2.5 mg/kg); (vii) hydroxytyrosol (2.5 mg/kg). These optimal doses were already established from our previous studies [20]. The white wine used in our study was Soave Doc Classico 2004 (La Rive) while the red wine was Reunite Lambrusco (Daunia). These wines were carefully selected based on their high content of tyrosol (17.06 mg/kg) and hydroxy tyrosol (2.69 mg/kg) in white wine and resveratrol (2.81 mg/L) in red wine.

Isolated working heart preparation

The animals were anesthetized with sodium pentobarbital (80 mg/kg, ip), (Abbott Laboratories, North Chicago, IL) and heparin sodium (500 IU/kg, iv) (Elkins-Sinn Inc., Cherry Hill, NJ) was used as an anticoagulant. The hearts perfused via working mode were subjected to 30 min ischemia followed by 2 h of reperfusion as described previously [21]. At the end of each experiment infarct size was measured by the triphenyltetrazolium chloride (TTC) staining method [22] or cardiomyocyte apoptosis was determined by the terminal deoxynucleotidyl transferase nick end labeling (TUNEL; Promega) method [22].

Western blot analysis

Tissues were homogenized in 1 ml buffer A (25 mM Tris-HCl, pH 8, 25 mM NaCl, 1 mM Na-orthovanadate, 10 mM NaF, 10 mM Na-pyrophosphate, 10 nM Okadaic acid, 0.5 mM EDTA, 1 mM PMSF, and 1×Protease inhibitor cocktail) in a Polytron homogenizer [23]. Homogenates were centrifuged at 2000 rpm at 4°C for 10 min and the nuclear pellet was resuspended in 500 µl of Buffer A with 0.1% Triton X-100 and 500 mM NaCl. Supernatant from the above centrifugation was further centrifuged at 10,000 rpm at 4°C for 20 min, and the resultant supernatant was used as cytosolic extract. The mitochondrial pellet was resuspended in 200–300 µl of Buffer A with 0.1% Triton X-100. The nuclei pellet and mitochondrial pellet were lysed by incubation for 1 h on ice with intermittent tapping. Homogenates were then centrifuged at 14,000 rpm at 4°C for 10 min, and the supernatant was used as nuclear lysate and mitochondrial lysate, respectively. Cytosolic, nuclear, and mitochondrial extracts were aliquoted, snap-frozen, and stored at -80°C till use. Total protein concentrations in cytosolic, nuclear, and mitochondrial extracts were determined using the BCA Protein Assay Kit (Pierce, Rockford, IL).

Proteins were separated in SDS-PAGE and transferred to nitrocellulose filters [23]. Filters were blocked in 5% nonfat dry milk and probed with primary antibody for overnight at 4°C. Primary antibodies such as SirT1, SirT3, SirT4, Pre B cell enhancing factor antibody (PBEF), histone, and glyceraldehyde-6-phosphate dehydrogenase (GAPDH) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA); whereas FoxO1, phospho-FoxO1 (Ser256), FoxO3a, phospho-FoxO3a (Ser253), and COX 4 were obtained from Cell Signaling Technology (Beverly, MA). All primary antibodies were used at the dilution of 1:1000. Protein bands were identified with horseradish peroxidase conjugated secondary antibody (1:2000 dilution) and Western Blotting Luminol Reagent (Santa Cruz Biotechnology). GAPDH, histone and COX 4 were used as loading control for cytosolic, nuclear, and mitochondrial protein, respectively.

Immunofluorescence staining

Formalin-fixed paraffin-embedded myocardial tissue sections were deparaffinized, and the antigen retrieval treatment was performed using 10 mM sodium citrate containing 0.05% Tween 20 at 90–95°C for 30 min. After washing with PBS, the slides were blocked with Powerblock (BioGenex, San Ramon, CA) for 10 min. Slides were washed with PBS and incubated with primary antibodies (rabbit anti-phospho-FoxO1; 1:50 dilution, and goat anti-SirT1; 1:100 dilution) in PBS containing 1% BSA for 2 h. Slides were washed and incubated with fluorescein-conjugated secondary antibodies (anti-rabbit Alexa Fluor 488, green, and anti-goat Alexa Fluor 594, red, both at 1:1000 dilutions) in the dark for 45 min. Nuclear staining was achieved with To-Pro 3 iodide (1:1000 dilution) for 45 min in the dark. The slides were washed and covered with mounting medium. Confocal microscopic images were obtained using a Zeiss LSM 510 (Thornwood, NY) confocal laser scanning microscope with 40×1.3 oil immersion objective by simultaneous recording in the 488 λ, 560 λ, and /or 615 λ channels as appropriate.

Results

Effects of white and red wines and their cardioprotective components on the expression of PBEF, SirTs, and FoxOs

We determined the induction of the proteins of PBEF, SirT1, SirT3, SirT4, FoxO1, and Foxo3a in the hearts of the rats after gavaging them with wines and their components for 14 days. The results are shown in Figs. 1–3. As shown in Fig. 1, PBEF is present even in the control heart, which remains the same in the ethanol-treated heart. As noted under Materials and methods, we gavaged a group of rats with ethanol equivalent to the amount present in the wines to exclude the possibility that the anti-aging protein expression is due to the alcohol present in wine. The amount of PBEF protein expression was highest for the white wine and resveratrol followed by tyrosol/hydroxytyrosol and red wine. In fact, the amount of PBEF in the red wine group was the same as that found in the control heart.

The induction of the expression of SirT proteins is shown in Figs. 2 and 3. All SirT proteins including SirT1, SirT3, and SirT4 are present in the control hearts. Interestingly, in ethanol-treated hearts, the expression of SirTs is reduced. SirT1 was increased almost to the same extent by all the treatments except for the tyrosol where the enhancement of SirT1 was lower compared to white wine/resveratrol/hydroxytyrosol/red wine. The enhancement of SirT3, on the other hand, was highest for both white and red wines followed by tyrosol and hydroxytyrosol/resveratrol. The increase of SirT4 was highest for white wine/resveratrol followed by hydroxytyrosol, tyrosol, and red wine.

FoxOs are inactivated through phosphorylation followed by their translocation and interaction with SirT1. FoxO1 is present in very small amount in the control heart, but alcohol treatment appreciably

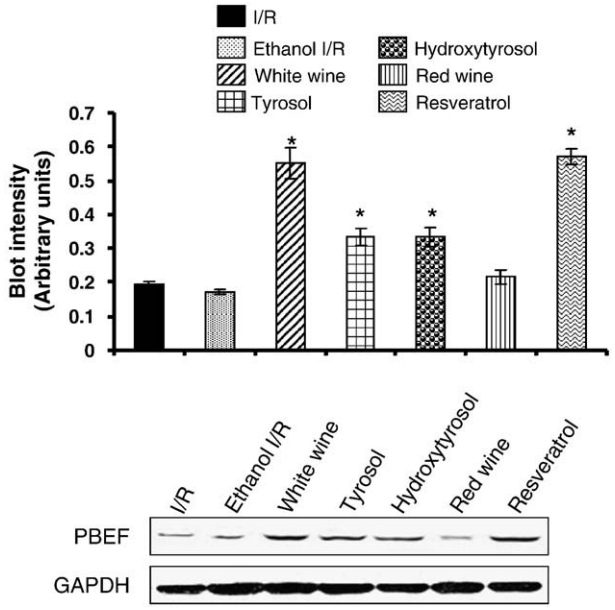

Fig. 1. Western blot analysis of PBEF in heart tissue obtained from control (I/R) and EtOH-, white wine-, tyrosol-, hydroxytyrosol-, red wine-, and resveratrol-treated heart. GAPDH was used as a loading control. Figures are representative images of three different groups. Results are expressed as mean±SEM of 3 groups of heart/group. *$p<0.05$ vs Ethanol I/R.

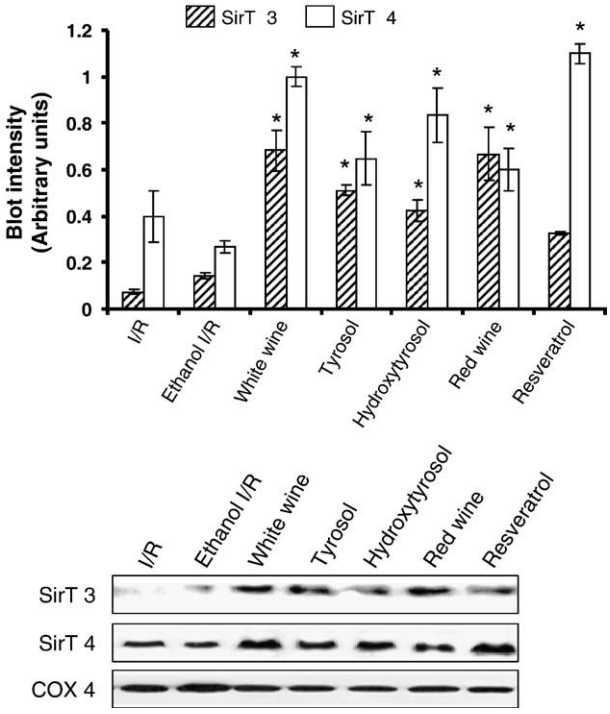

Fig. 3. Western blot analysis of SirT3 and SirT4 protein in heart tissue obtained from control (I/R) and EtOH-, white wine-, tyrosol-, hydroxytyrosol-, red wine-, resveratrol-treated hearts. COX 4 was used as a loading control. Figures are representative images of three different groups, and each experiment was repeated at least thrice. Results are expressed as mean±SEM of 3 groups of heart/group. *$p<0.05$ vs Ethanol I/R.

increased its induction (Fig. 4). White wine also induced the expression of FoxO1 to some extent followed by red wine. No induction was found for tyrosol, hydroxytyrosol, and resveratrol. Both white and red wines as well as tyrosol and resveratrol significantly increased the phosphorylation of FoxO1, while hydroxytyrosol increased the phosphorylation minimally. FoxO3a was not induced by any of the wines or its components. However, white wine and resveratrol induced the phosphorylation of FoxO3a followed by tyrosol, red wine, and hydroxytyrosol.

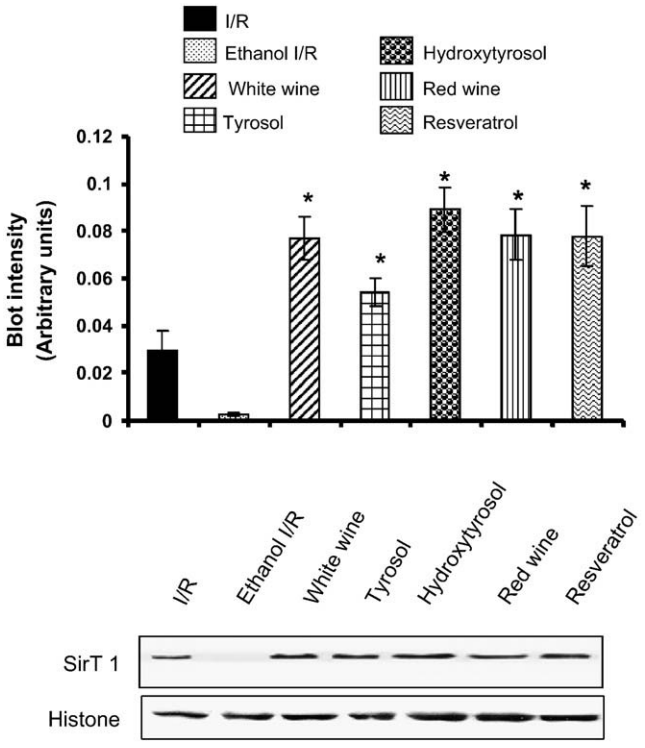

Fig. 2. Western blot analysis of SirT1 protein in heart tissue obtained from control (I/R) and EtOH-, white wine-, tyrosol-, hydroxytyrosol-, red wine-, resveratrol-treated hearts. Histone was used as a loading control. Figures are representative images of three different groups, and each experiment was repeated at least thrice. Results are expressed as mean±SEM of 3 groups of heart/group. *$p<0.05$ vs Ethanol I/R.

In order to confirm the results of our Western blotting, we performed immunofluorescence staining with phospho-FoxO1- and SirT1-specific antibodies as noted under Materials and methods. We found that treatment with white wine, tyrosol, hydroxytyrosol, red wine, and resveratrol induces the nuclear expression of SirT1 and phospho-FoxO1 (Fig. 5).

Cardioprotection with wines and their components

To examine the cardioprotection exerted by the wines and their components, we determined myocardial infarct size and cardiomyocyte apoptosis. The control heart displayed about 40% infarct size, consistent with previous results (Table 1). Both red and white wines and their components reduced the amount of infarct size in the following order: resveratrol>hydroxytyrosol>red wine>white wine-tyrosol. Cardiomyocyte apoptosis determined by TUNEL staining showed a similar pattern.

Discussion

There are several salient features in the present study: (i) the first, not only did white wine induce the longevity proteins including SirT1, SirT3, and SirT4 and the phosphorylation of FoxO1 and FoxO3a in the heart, the amounts of the induced proteins were higher than those induced by red wine or resveratrol; (ii) the ability to induce anti-aging proteins followed an order: white wine>resveratrol>tyrosol>red wine/hydroxytyrosol; (iii) the abilities to protect the heart from ischemia/reperfusion injury followed an order resveratrol>hydroxytyrosol>red wine>white wine>tyrosol. Previously, we showed that the pattern of Akt activation by phosphorylation and activation of Bcl-2 followed the same pattern [20],

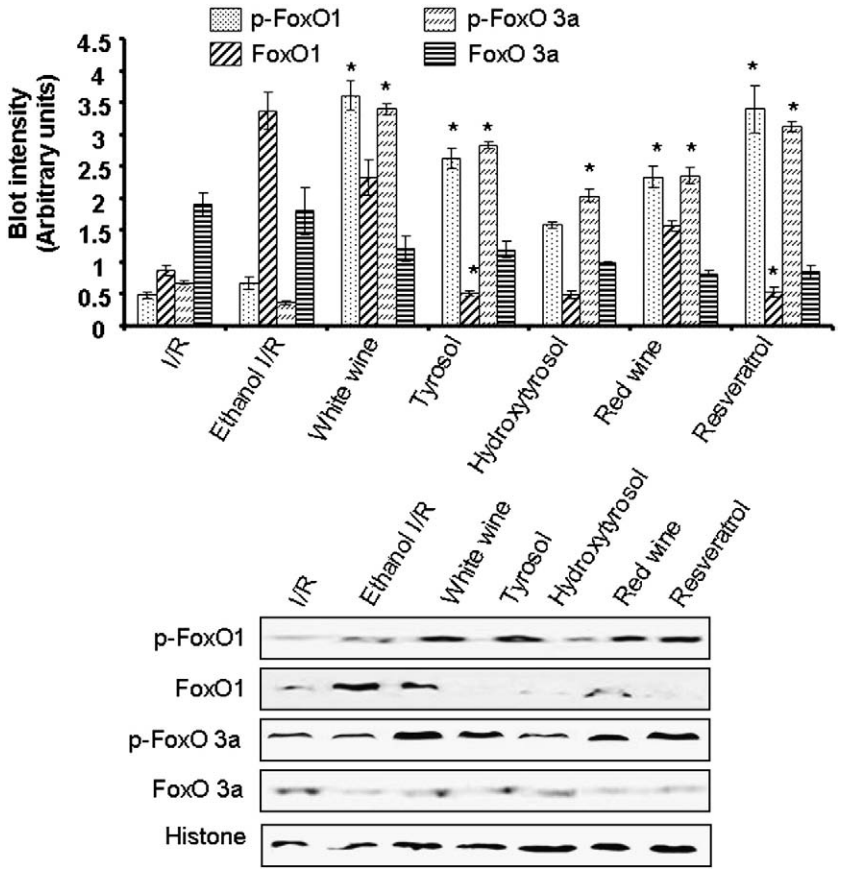

Fig. 4. Western blot analysis of phospho-FoxO1, FoxO1, phospho-FoxO3a, and FoxO3a protein in heart tissue obtained from control (I/R) and EtOH-, white wine-, tyrosol-, hydroxytyrosol-, red wine-, resveratrol-treated hearts. Histone was used as a loading control. Figures are representative images of three different groups, and each experiment was repeated at least thrice. Results are expressed as mean ± SEM of 3 groups of heart/group. *$p < 0.05$ vs Ethanol I/R.

suggesting that the survival signaling pathway and cardioprotection followed identical pathways. This would indicate while all of the tested compounds induced anti-aging/longevity proteins and provided cardioprotection, their molecular mechanism appear to be different.

It is not surprising that resveratrol and red wine induced the expression of SirT1 protein. However, to the best of our knowledge, no study has ever been performed demonstrating resveratrol's ability to induce several SirT-related proteins. The results of our study showed that resveratrol induced the activation of SirT1, SirT3, and SirT4, and the phosphorylation of FoxO1 and FoxO3a as well as PBEF proteins. The activation of SirT1 with resveratrol has been known for some time, and accordingly resveratrol is known to prolong life span [24]. Mammalians possess seven SirTs (SirT1–7) that occupy different subcellular compartments including nucleus (SirT1, -2, -6, -7), cytoplasm (SirT1 and SirT2), and the mitochondria (SirT3, -4, and -5). SirT1, the first member of the Sirtuin family, is a NAD^+-dependent histone deacetylase that plays a crucial role in chromatin remodeling associated with gene silencing and prolongation of life span [25]. SirT1 regulates several transcription factors including FoxO1, which is inactivated by phosphorylation via Akt [26]. Our results also showed the phosphorylation of FoxO1 along with the activation of SirT1. SirT3 and 4 are localized in mitochondria where they regulate aging and energy metabolism. Similar to SirT1, SirT3 is also highly expressed in the heart [27]. SirT3 remains in an inactive form, and following signal peptide cleavage it becomes active as histone deacetylase [27]. However, the molecular target of SirT3 remains unknown. SirT4 is ubiquitously present in all tissues including heart, but its biological role is not known. Thus, the significance of activation of SirT3 and SirT4 is not clear in the present study.

The phosphorylation of FoxO1 and FoxO3a in conjunction with SirT raises the possibility that these FoxOs are direct substrates for SirTs in the heart activated by resveratrol. In mammalian heart, there are four evolutionarily conserved FoxOs—FoxO1, FoxO3, FoxO4, and FoxO6. Besides many functions being regulated by FoxOs, they induce cell death via activating the transcription of some proapoptotic genes [28]. Nutritional deprivation also leads to the phosphorylation of both FoxO1 and FoxO3a similar to white and red wines and their cardioprotective components, as they play a role in promoting adaptation to fasting [29]. It is for this reason that we examined the phosphorylation of both FoxO1 and FoxO3a in our study. FoxO1 phosphorylation is influenced by acetylation/deacetylation reactions. SirT1 binds directly to FoxO1 and catalyzes its deacetylation, thereby modulating FoxO1 transcriptional activity [30]. Insulin induces phosphoinositol-3 kinase/Akt-dependent phosphorylation of FoxO, facilitating its interaction with 14-3-3 protein, resulting in nuclear exclusion followed by proteasomal degradation [31]. In the present study, resveratrol and tyrosol as well as red and white wines all activated SirT1, SirT3, and SirT4, and further induced the phosphorylation of both FoxO1 and FoxO3 in the nucleus.

PBEF is a nicotinamide phosphoribosyl transferase, catalyzing the rate-limiting step in the biosynthesis of NAD^+ [32], and contributing to the cellular pathway of aging and longevity [33]. PBEF thus supplies NAD^+ to SirT1, which is a NAD-dependent HDAC. Once activated by PBEF, SirT1 promotes cell survival. While resveratrol has already been found to activate such cell survival, our results demonstrate that red wine, white wine, tyrosol, and hydroxytyrosol also can activate the survival and longevity.

In the present study, resveratrol and tyrosol as well as red and white wines all activated SirT1, SirT3, and SirT4, and further induced

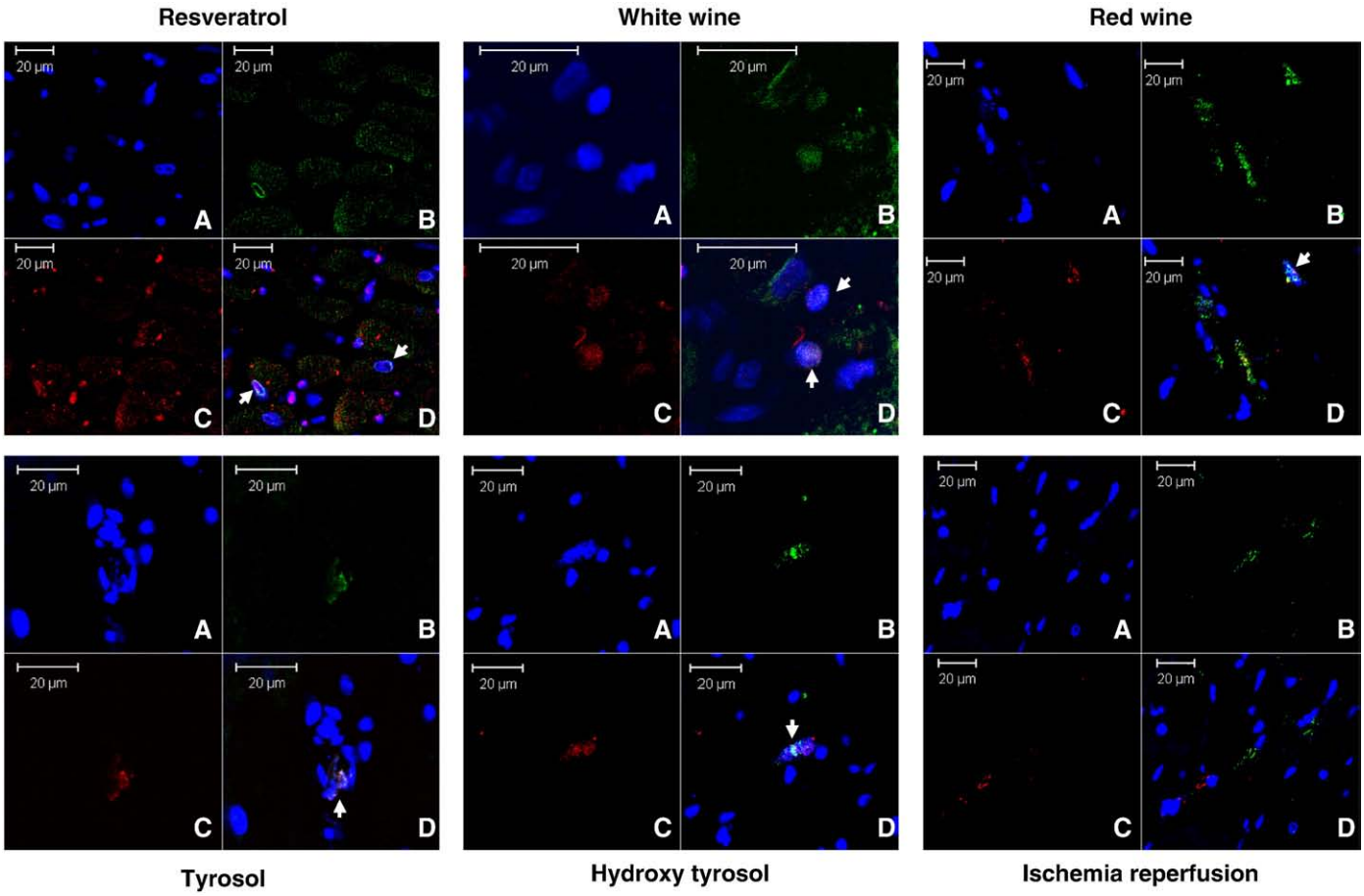

Fig. 5. Confocal microscopic images of formalin-fixed paraffin embedded myocardial tissue sections stained with (A) nuclear stain (blue channel; Topro-3 iodide), (B) p-FoxO (green channel; Alexa Fluor 488), and (C) SirT1 (red channel; Alexa Fluor 594). (D) Merging of images A, B, and C. Arrows in D indicate the coexpression (white) of p-FoxO1 and SirT1 in the nucleus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the phosphorylation of both FoxO1 and FoxO3a in the nucleus. To the best of our knowledge, other compounds present in wines, e.g., catechins, flavonols, and sulfites do not possess the ability to induce these survival proteins. However, wines contain many other polyphenolic compounds, and it may be possible that some of them possess the ability to induce these longevity genes.

Transcriptional activities of FoxO transcription factors including FoxO1, FoxO3a, and FoxO4 are repressed by the binding of SirT1, which deacetylates FoxOs [11]. Binding of SirT1 may downregulate the activity of FoxOs by destabilizing the protein, decreasing its DNA binding activity, or changing protein–protein interactions, as suggested for other transcription factors [34]. During the course of activation, FoxO gets acetylated and interact with coactivator p300 leading to apoptosis in response to withdrawal of growth factors, where the phosphorylation of FoxO by erythropoietin prevents acetylation and their interaction with coactivator p300 in erythroid progenitor cells [35]. In the present study, we found that the nuclear expression of phosphorylated FoxO1 and FoxO3a and the activation of SirT1 in the nucleus are increased in wine and wine component-treated groups compared to vehicle-treated control groups. Moreover, our confocal microscopic images show the coexpression of phosphorylated FoxO1 and SirT1 in the nucleus. It is intriguing to know whether phosphorylation of FoxO by Akt followed by binding of SirT1 with FoxO or binding of SirT1 with FoxO could induce phosphorylation of FoxO by Akt. Further studies are required to clarify the above question.

As noted earlier, our results demonstrated that the most potent activator of anti-aging/longevity proteins (SirTs, FoxOs, PBEF) were white wine followed by resveratrol, tyrosol, red wine, and

Table 1
Effect of ethanol, white wine, tyrosol, hydroxytyrosol, red wine, and resveratrol on the infarct size determined by TTC and cardiomyosite apoptosis determined by the TUNEL method

	I/R	Ethanol	White wine	Tyrosol	Hydroxy-tyrosol	Red wine	Resveratrol
Infarct size	37±2	31±1.5	24±2*	26±2*	21±1*	23±1.5*	20±1*
Apoptosis	38±2	28±1.5	19±1*	14±1*	12±2*	15±1*	14±2*

Results are expressed as mean±SEM of 3 groups of heart/group.
* $p<0.05$ vs Ethanol I/R.

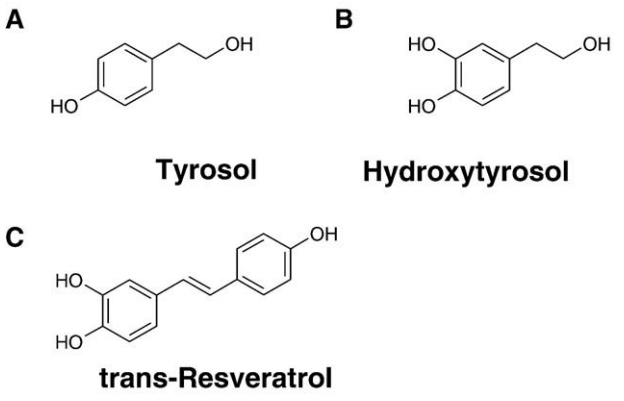

Fig. 6. Structure of resveratrol, tyrosol, and hydroxytyrosol.

hydroxytyrosol while the most effective cardioprotection was achieved with resveratrol followed by hydroxytyrosol>red wine-white wine>tyrosol. This clearly suggests that the ultimate survival pathway triggered by Akt phosphorylation is likely to be different from the anti-aging pathway FoxO-Akt. We and others have shown that resveratrol potentiates cardioprotection by activating the PI-3-kinase/Akt signaling pathway [36]. Consistent with these findings, the cardioprotective abilities of the test compounds as evidenced by reduced myocardial infarct size and cardiomyocyte apoptosis followed an identical pattern to that for Akt activation resveratrol>red wine>hydroxytyrosol>tyrosol>white wine [20]. The similarities of the mechanism of action among resveratrol, tyrosol, and hydroxy tyrosol may be attributed to the structural similarities among these three compounds. The phenolic structure shared by all three compounds is likely to influence the antioxidant activity due to steric factors as well as those related to position and type of functional groups on the phenol ring. Antioxidant activity of the biophenols depends on the number of hydroxyl group in the molecule. All three molecules possess one phenol group. Resveratrol and hydroxytyrosol contain one extra -OH group in the same ring. All the three compounds have a side chain in the para position, and in the case of tyrosol and hydroxytyrosol, this side chain is one hydroxyl ethyl group whereas in the case of resveratrol instead of -OH group there is one phenol group. Moreover in the side chain there is an unsaturated bond (Fig. 6). The results of the present study indicate that the ability to induce longevity and/or survival signal does not depend on the number of hydroxyl groups.

In conclusion our results show the ability of red wine, white wine, and its components tyrosol and hydroxytyrosol to induce several related longevity/anti-aging proteins including PBEF, SirTs, and FoxOs similar to resveratrol.

Acknowledgments

This study was supported in part by NIH HL 22559, HL33889, and HL34360.

References

[1] Miyazaki, R.; Ichiki, T.; Hashimoto, T.; Inanaga, K.; Imayama, I.; Sadoshima, J.; Sunagawa, K. SIRT1, a longevity gene, downregulates angiotensin II type 1 receptor expression in vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* **28:**1263–1269; 2008.
[2] Borra, M. T.; Smith, B. C.; Denu, J. M. Mechanism of human SIRT1 activation by resveratrol. *J. Biol. Chem.* **280:**17187–17195; 2005.
[3] Kaeberlein, M.; McDonagh, T.; Heltweg, B.; Hixon, J.; Westman, E. A.; Caldwell, S. D.; Napper, A.; Curtis, R.; DiStefano, P. S.; Fields, S.; Bedalov, A.; Kennedy, B. K. Substrate-specific activation of Sirtuins by resveratrol. *J. Biol. Chem.* **280:**17038–17045; 2005.
[4] Jiang, W. J. Sirtuins: novel targets for metabolic disease in drug development. *Biochem. Biophys. Res. Commun.* **373:**341–344; 2008.
[5] Guarente, L. Sirtuins in aging and disease. *Cold Spring Harb. Symp. Quant. Biol.* **72:**483–488; 2007.
[6] Griswold, A. J.; Chang, K. T.; Runko, A. P.; Knight, M. A.; Min, K. T. Sir2 mediates apoptosis through JNK-dependent pathways in Drosophila. *Proc. Natl. Acad. Sci. USA* **105:**8673–8678; 2008.
[7] Gruber, J.; Tang, S. Y.; Halliwell, B. Evidence for a trade-off between survival and fitness caused by resveratrol treatment of *Caenorhabditis elegans*. *Ann. N. Y. Acad. Sci.* **100:**530–542; 2007.
[8] Terzibasi, E.; Valenzano, D. R.; Cellerino, A. The short-lived fish *Nothobranchius furzeri* as a new model system for aging studies. *Exp. Gerontol.* **42:**81–89; 2007.
[9] van der Veer, E.; Nong, Z.; O'Neil, C.; Urquhart, B.; Freeman, D.; Pickering, J. G. Pre-B-cell colony-enhancing factor regulates NAD+-dependent protein deacetylase activity and promotes vascular smooth muscle cell maturation. *Circ. Res.* **97:**25–34; 2005.
[10] Michishita, E.; Park, J. Y.; Burneskis, J. M.; Barrett, J. C.; Horikawa, I. Evolutionarily conserved and non-conserved cellular localizations and functions of human SIRT proteins. *Mol. Biol. Cell* **10:**4623–4635; 2005.
[11] Motta, M. C.; Divecha, N.; Lemieux, M.; Kamel, C.; Chen, D.; Gu, W.; Bultsma, Y.; McBurney, M.; Guarente, L. Mammalian SIRT1 represses forkhead transcription factors. *Cell* **116:**551–563; 2004.
[12] Lam, E. W.; Francis, R. E.; Petkovic, M. FOXO transcription factors: key regulators of cell fate. *Biochem. Soc. Trans.* **34:**722–726; 2006.
[13] Karen, C.; William, A.; Biggs, H. Regulation of the FoxO family of transcription factors by phosphatidylinositol-3 kinase-activated signaling. *Arch. Biochem. Biophys.* **403:**292–298; 2002.
[14] Van Der Heide, L. P.; Hoekman, M. F.; Smidt, M. P. The ins and outs of FoxO shuttling: mechanisms of FoxO translocation and transcriptional regulation. *Biochem. J.* **380:**297–309; 2004.
[15] Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **341:**454–457; 1993.
[16] Bertelli, A. A. E.; 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.* **957:**295–301; 2002.
[17] Vázquez-Agell, M.; Sacanella, E.; Tobias, E.; Monagas, M.; Antúnez, E.; Zamora-Ros, R.; Andrés-Lacueva, C.; Lamuela-Raventós, R. M.; Fernández-Solá, J.; Nicolás, J. M.; Estruch, R. Inflammatory markers of atherosclerosis are decreased after moderate consumption of cava (sparkling wine) in men with low cardiovascular risk. *J. Nutr.* **137:**2279–2284; 2007.
[18] Williams, M. J. A.; Sutherland, W. H. F.; Whelan, A. P.; McCormick, M. P.; de Jong, S. A. Acute effect of drinking red and white wines on circulating levels of inflammation-sensitive molecules in men with coronary heart disease. *Metabolism* **53:**318–323; 2004.
[19] Cui, J.; Tosaki, A.; Bertelli, A. A.; Bertelli, A.; Maulik, N.; Das, D. K. Cardioprotection with white wine. *Drugs Exp. Clin. Res.* **28:**1–10; 2002.
[20] Dudley, J. I.; Lekli, I.; Mukherjee, S.; Das, M.; Bertelli, A. A.; Das, D. K. Does white wine qualify for French paradox? Comparison of the cardioprotective effects of red and white wines and their constituents: resveratrol, tyrosol, and hydroxytyrosol. *J. Agric. Food Chem.* **56:**9362–9373; 2008.
[21] Engelman, D. T.; Watanabe, M.; Engelman, R. M.; Rousou, J. A.; Kisin, E.; Kagan, V. E.; Maulik, N.; Das, D. K. Hypoxic preconditioning preserves antioxidant reserve in the working rat heart. *Cardiovasc. Res.* **29:**133–140; 1995.
[22] Mukherjee, S.; Gangopadhyay, H.; Das, D. K. Broccoli: a unique vegetable that protects mammalian hearts through the redox cycling of the thioredoxin superfamily. *J. Agric. Food Chem.* **56:**609–617; 2008.
[23] Mukherjee, S.; Lekli, I.; Das, M.; Azzi, A.; Das, D. K. Cardioprotection with alpha-tocopheryl phosphate: amelioration of myocardial ischemia reperfusion injury is linked with its ability to generate a survival signal through Akt activation. *Biochim. Biophys. Acta* **9:**498–503; 2008.
[24] Baxter, R. A. Anti-aging properties of resveratrol: review and report of a potent new antioxidant skin care formulation. *J. Cosmet. Dermatol.* **7:**2–7; 2008.
[25] John, M. Linking chromatin function with metabolic networks: Sir2 family of NAD^+-dependent deacetylases. *Trends Biochem. Sci.* **28:**41–48; 2003.
[26] Brunet, A.; Sweeney, L. B.; Sturgill, J. F.; Chua, K. F.; Greer, P. L.; Lin, Y.; Tran, H.; Ross, S. E.; Mostoslavsky, R. E.; Cohen, H. Y.; Hu, L. S.; Cheng, H. L.; Jedrychowski, M. P.; Gygi, S. P.; Sinclair, D. A.; Alt, F. W.; Greenberg, M. E. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* **303:**2011–2015; 2004.
[27] Onyango, P.; Celic, I.; McCaffery, J. M.; Boeke, J. D.; Fein, A. P. SIRT3, a human SIR2 homologue, is an NAD-dependent deacetylase localized to mitochondria. *Proc. Natl. Acad. Sci. USA* **99:**13653–13658; 2002.
[28] Carter, M. E.; Brunet, A. FOXO transcription factors. *Curr. Biol.* **17:**R113–R114; 2007.
[29] Furuyama, T.; Kitayama, K.; Yamashita, H.; Mori, N. Forkhead transcription factor FOXO1 (FKHR)-dependent induction of PDK4 gene expression in skeletal muscle during energy deprivation. *Biochem. J.* **375:**365–371; 2003.
[30] Nakae, J.; Cao, Y.; Daitoku, H.; Fukamizu, A.; Ogawa, W.; Yano, Y.; Hayashi, Y. The LXXLL motif of murine forkhead transcription factor FoxO1 mediates SirT1-dependent transcriptional activity. *J. Clin. Invest.* **116:**2473–2483; 2006.
[31] Matsuzaki, H.; Daitoku, H.; Hatta, M.; Tanaka, K.; Fukamizu, A. Insulin-induced phosphorylation of FKHR (Foxo1) targets to proteasomal degradation. *Proc. Natl. Acad. Sci. USA* **100:**11285–11290; 2003.
[32] Ziegler, M. New functions of a long known molecule. Emerging roles of NAD in cellular signaling. *Eur. J. Biochem.* **267:**1550–1564; 2000.
[33] Revollo, J. R.; Grimn, A. A.; Imai, S. The NAD biosynthesis pathway mediated by nicotinamide phosphoribosyltransferase regulates SirT2 activity in mammalian cells. *J. Biol. Chem.* **279:**50754–50763; 2004.
[34] Brooks, C. L.; Gu, W. Ubiquitination, phosphorylation and acetylation: the molecular basis for p53 regulation. *Curr. Opin. Cell Biol.* **15:**164–171; 2003.
[35] Mahmud, D. L.; G-Amlak, M.; Deb, D. K.; Platanias, L. C.; Uddin, S.; Wickrema, A. Phosphorylation of forkhead transcription factors by erythropoietin and stem cell factor prevents acetylation and their interaction with coactivator p300 in erythroid progenitor cells. *Oncogene* **21:**1556–1562; 2002.
[36] Das, S.; Fraga, C. G.; Das, D. K. Cardioprotective effect of resveratrol via HO-1 expression involves p38 map kinase and PI-3-kinase signaling, but does not involve NFkappaB. *Free Radic. Res.* **40:**1066–1075; 2006.