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Role of thioredoxin-1 in ischemic preconditioning, postconditioning and aged ischemic hearts

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ABSTRACT

Thioredoxin is one of the most important cellular antioxidant systems known to date, and is responsible of maintaining the reduced state of the intracellular space. Trx-1 is a small cytosolic protein whose transcription is induced by stress. Therefore it is possible that this antioxidant plays a protective role against the oxidative stress caused by an increase of reactive oxygen species concentration, as occurs during the reperfusion after an ischemic episode. However, in addition to its antioxidant properties, it is able to activate other cytoplasmic and nuclear mediators that confer cardioprotection. It is remarkable that Trx-1 also participates in myocardial protection mechanisms such as ischemic preconditioning and postconditioning, activating proteins related to cellular survival. In this sense, it has been shown that Trx-1 inhibition abolished the preconditioning cardioprotective effect, evidenced through apoptosis and infarct size. Furthermore, ischemic postconditioning preserves Trx-1 content at reperfusion, after ischemia. However, comorbidities such as aging can modify this powerful cellular defense leading to decrease cardioprotection. Even ischemic preconditioning and postconditioning protocols performed in aged animal models failed to decrease infarct size. Therefore, the lack of success of antioxidants therapies to treat ischemic heart disease could be solved, at least in part, avoiding the damage of Trx system.

1. Introduction

Trx-1[1] is one of the molecules with the highest antioxidant effect known to date [1], and keeps the intracellular compartment in reduced state [1,2]. In 1964 for first time a protein, called thioredoxin, was purified from *Escherichia coli B*. This protein in the presence of reduced triphosphopyridine nucleotide can replace reduced lipoate as the hydrogen donor in the reductive formation of deoxycytidine diphosphate from CDP[2] with the CDP-reductase system from *Escherichia coli* [3]. In 1975 Holgrem et al. describes for the first time its structure [4]. This antioxidant enzyme has a long evolutionary history and was found in a large number of organisms, from *Archae* to mammals, and it is present in all cells, including myocytes [5].

Organisms have developed a wide specialized subset of Trx proteins, each localized in different cellular compartments. Some Trx are abundant in the cytosol, while others are translocated into the nucleus or mitochondria. They can also be found associated with the cell membrane or secreted into the extracellular compartment [2]. The antioxidant function of this enzyme is due to a dithiol group in its conserved active site (-Cys-Gly-Pro-Cys-) located in the Trx surface, in a short amino-terminal segment. This allows it to reduce thiol groups in several redox-sensitive proteins. By this way, Trx acts on oxidized (and inactive) proteins, reducing them and restoring their functionality. Also, it is important to mention that Trx is part of a system that requires Trx-R[3] and reduced NADPH[4] to function properly [1,2]. The Trx-R enzyme is responsible for reducing the oxidized Trx active site, and consequently uses NADPH as

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[1] Thioredoxin-1
[2] Cytidine diphosphate
[3] Thioredoxin reductase.
[4] Nicotinamine adenine dinucleotid phosphate.

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an electron donor. Thus, Trx is again active and it can continue with its reducing functions [1]. This system is completed with Trx peroxidase, which transforms hydrogen peroxide into water.

In mammals particularly, the most studied protein in the Trx family is Trx-1, a small 12 kDa[5] protein containing cysteine at positions 32 and 35 of its active site [1,2,6]. This protein is ubiquitous of the cellular cytoplasm, where it fulfills its antioxidant function, but under stress conditions it is able to translocate to the nucleus and regulate several transcription factors such as NFκB,[6] protein 1 activator and p53 [6].

Trx-1 activity can be regulated by several mechanisms, most notably in its expression levels, location, interaction with other molecules, and post-translational modifications. Moreover, an important feature of this protein is that the Trx gene promoter region contains a number of stress response elements. Hence, Trx could transcript by several stimuli such as TNFα,[7] H_2O_2,[8] UV,[9] ischemia and thermal shock [2]. In addition, Trx-1 can suffer post-translational modifications such as *S*-nitrosylation, oxidation and nitration. *S*-nitrosylation has beneficial effects since it enhances Trx-1 activity, and therefore decreases apoptosis by inactivation of caspase-3 [7]. Oxidation and nitration produce partial or total Trx-1 inactivation, respectively, resulting in the attenuation of its biological functions [7].

It is important to remark that Trx-1 acts as a reductase in the redox control, protecting aggregation and oxidative inactivation proteins, and it also helps to maintain the cell redox homeostasis [1,2,5] that could be damage by ROS,[10] which is underlying in the pathophysiology of many cardiovascular diseases. It also fulfills an important role in the I/R[11] injury, in the regulation of programmed death by denitrosylation, and also acts as a growth factor, modulating inflammatory response and promoting protein folding [5].

2. Role of thioredoxin-1 in the ischemia/reperfusion injury

Since Trx-1 is a transcription protein induced by stress, it is possible that this antioxidant fulfills a protective role against stress by ROS overload in I/R injury. This has been demostrated in both *in vivo* and *in vitro* animal models, where Trx-1 has specific beneficial effects in the heart, including I/R cardioprotection reducing infarct size and improving ventricular function recovery [8]. Furthermore, it was demonstrated that Trx-1 administration protects the heart against I/R injury, through adenovirus mediated gene transfection, increasing cell survival [9]. In relation to this effect, Mitsui et al. [10] showed that Trx-1 over-expression in mice prolonged lifespan by 35% compared to Wt[12] mice. In a similar manner, Shijoi et al. [6] used transgenic mice expressing increased levels of human Trx-1 showed that mitochondria, myofibrils, and other cellular details were much better maintained in ADR[13]-treated Trx-1-TG[14] mice than in ADR-treated non transgenic (Wt) mice. The increase in the protein carbonyl content, a marker of cellular protein oxidation, was suppressed in ADR-treated Trx-1-TG mice compared with ADR-treated Wt mice. The formation of hydroxyl radicals in ADR-treated heart homogenates of Trx-1-TG mice was decreased compared with Wt mice. For the survival study, all Wt mice treated with ADR died within 6 weeks, but 5 of 6 Trx-1-TG mice treated with ADR survived more than 8 weeks. These findings suggest that Trx-1 fulfills an important role in cardiomyocyte cellular defense against ROS damage. Furthermore, Kaga et al. [11] demonstrated that treatment with resveratrol induces Trx-1, and vascular endothelial growth factor in infarcted hearts, resulting in infarct size reduction and in cardiac function improvement. This would suggest that Trx-1 regulates gene transcription by interacting with several transcription factors increasing antioxidant, antiapoptotic and pro-angiogenic properties, depending on cellular conditions. In an *in vivo* model it was demonstrated that human Trx-1 administration inhibits apoptosis and infarct size in myocardial tissue subjected to I/R [12]. The underlying mechanism appears to be partially dependant on p38 reduced kinase activation [12]. In a similar manner, regarding I/R injury, Aota et al. [13] demonstrated that the administration of recombinant human Trx reduced the incidence of reperfusion arrhythmias. Nakamura et al. [14] showed, in patients subjected to bypass surgery, that Trx inactivation was a deleterious mechanism in I/R injury. Kihlström et al. [15] demonstrated that endurance training by swimming showed cardioprotection reducing oxidative stress with a concomitant increase in Trx reductase (active form). Similarly, Tao et al. [16] showed that administration of Trx-1 in vivo exerts significant protective effects on myocardial apoptosis decreasing myocardial infarct size, by inhibiting p38-MAPK activation. Considering the aforementioned studies, all findings show that during ischemia and reperfusion, the endogenous activity of the Trx-1 system is decreased. These results strongly suggest that cell damage that occurs in myocardial infarction seems to be due to reactive oxygen species production. Thus, the increase of the Trx-1 protein activity would protect from injury by myocardial ischemia and reperfusion.

PTEN[15] is one of the proteins that could explain the protection conferred by Trx-1 in I/R injury, because it has been shown that Trx1 inhibits PTEN [17]. This mediator avoids activation of PI3K/Akt complex that confers protection against I/R injury [18–20]. Furthermore, there are several studies relating PTEN with Akt and oxidative stress in different pathologies [18–21]. These studies show that PTEN inhibition by phosphorylation, ROS, or binding with other proteins such as Trx-1, activate Akt pro-survival pathway and promote cell survival [19,20,22].

Considering the aforementioned studies, we can partially conclude that Trx-1 plays a key role in the cells maintaining the redox balance in normal conditions. Also, in pathological conditions Trx-1 is able to prevent the damage caused by oxidative stress when its intracellular concentration is increased, as in the case of over-expression models, or if Trx-1 inactivation is prevented.

3. Thioredoxin effects on cardioprotection mechanisms. Ischemic preconditioning and postconditioning

It is widely known that in endogenous protection mechanisms, PC[16] and PostC[17] increase cell survival pathways, at least partially, by decreasing the oxidative stress damage after I/R injury [23–26]. Therefore it is interesting to review the role of the Trx system in the endogenous protection mechanisms.

3.1. Ischemic preconditioning

In 1986, Murry et al. [27] observed that the infarct size resulting from a 40 min of ischemia produced by the occlusion of the anterior descendant coronary artery in dogs, could be reduced if the heart was subjected to four brief ischemic episodes

[5] Kilo-Dalton.
[6] Nuclear kappa factor B.
[7] Tumor necrosis factor alpha.
[8] Hydrogen peroxide.
[9] Ultraviolet rays.
[10] Reactive oxygen species.
[11] Ischemia/reperfusion.
[12] Wild type.
[13] Adriamycin.
[14] Transgenic.
[15] Phosphatase and tensin homolog.
[16] Ischemic preconditioning.
[17] Ischemic postconditioning.

followed by 5 of reperfusion prior to prolonged ischemia. This powerful physiological cardioprotective mechanism was termed ischemic preconditioning, and was demonstrated in several species including humans [27–29]. PC is mediated by the adenosine receptors activation [30–32]. Nowadays, it is known that during brief episodes of ischemia, many chemical mediators are released by the myocardium, including: adenosine, noradrenaline, bradikinine, opioids and endothelin [30,33–35]. All these agents could bind myocyte membrane receptors and contribute to the activation of the PC via coupling their receptors to Gi protein. In relation to adenosine and PC, it was also evidenced that the A3 receptor preconditions human [36] and rabbit [37] hearts. Cohen et al. [38] demonstrated that adenosine receptor couples to Gi protein, leading to DAG[18] and IP3[19] formation through PKC[20] phosphorylation. This way, and through different intermediates, mitochondrial K^+_{ATP} channels open and release ROS from the mitochondria [38]. These small amounts of ROS activate PKC and downstream kinases, which participate in the PC cardioprotection. Tyrosine-kinases phosphorylate tyrosine residues of certain proteins and acts as mediators in many intracellular signal transduction events [39–42]. Different studies suggest that ROS are not only deleterious, but are also essential for the biology and physiology of myocytes [42,43]. Therefore, depending on the myocyte antioxidant reserve amount, ROS can either be destroyed or persist. In case of persistence they can fulfill the role of a second messenger or signaling molecule after an ischemic episode [42–45].Taken together, the cardioprotective abilities of PC appear to be linked to redox signaling [44,45] and it is in this sense where the antioxidant systems, such as Trx-1, play an important role in conferred cardioprotection.

Since it is known that one of the main triggers for the Trx-1 is the increased oxidative stress that occurs during an I/R episode [46–48], it is not surprising that Trx-1 levels decreased after ischemia. Release of Trx-1 is likely due to the adaptive response elicited by PC, and it is important to note that these results are consistent with previous observations demonstrating that Trx-1 is inducible by stress [49]. Thus, as we mentioned several stimulus can induce the increase of Trx-1 as part of the cell defense capabilities [6,46]. Consistent with these results, Trx-1 inhibition, abolished the PC cardioprotective effect, evidenced through apoptosis and infarct size [8]. In a similar manner, Chiueh et al. [50] showed that PC increases human Trx mRNA and protein for cytoprotection. Furthermore, cytosolic Trx-1 and mitochondrial Trx-2 suppress free radical formation, lipid peroxidation, oxidative stress, and mitochondria-dependent apoptosis and thus, to minimize the role of either Trx-1 or Trx-2 is detrimental to cell survival [50]. In conclusion, preconditioning adaptation and/or small and brief amounts of ROS induce a delayed nitric oxide-mediated compensatory mechanism for cell survival and vitality in the cardiovascular system.

Due to all the aforementioned, we can conclude that Trx-1 can play an essential role in the adaptation process of I/R injury, given that Trx-1 regulates the transcription of many genes that are also regulated by PC [51]. This way, Trx can regulate the "redox switch" changing the I/R death signal into a survival cell signal mediated by PC [52] triggering an adaptive response and being able to use the myocytes own defense.

3.2. Ischemic postconditioning

It has been shown that not only PC can be effective in cardioprotection against I/R injury. Also brief episodes of ischemia/reperfusion performed at the onset of reperfusion (PostC) reduced infarct size [53]. It has been suggested that PostC is effective as PC in limiting infarct size and preserving post ischemic endothelial function [53]. Given that PC stimulus is performed before ischemia, the concept of ischemic PostC may be more useful than PC in the clinical setting. In the PostC original paper, the authors suggested that the mechanisms by which both PC and PostC confer protection would be different, given that PC stimulates signals that activate mechanisms prior to ischemia, whereas in PostC they would take place during reperfusion. Subsequent data of Yellon et al. [54] suggested that this hypothesis would not be correct. They demonstrated that the inhibition of the PI3K[21], enzyme and the MEK1/2[22] during the first 15 min of reperfusion, abolishes the PostC protective effect [54]; and it is well known that these proteins are involved in cardioprotection conferred by PC. Later, Tsang et al. [54] and Yang et al. [55] demonstrated the important role of the PI3K-AKT and MEK1/2/Erk complexes in the PostC protection mechanisms. Subsequent studies showed that PostC was effective in different species such as rabbit [56] and rat [54] *in vivo*, isolated rabbit [55] and rat [57] hearts, isolated cardiomyocytes [58] and human cardiomyocytes [59]. Kin et al. [60] demonstrated that ischemic postconditioning was also effective in rats. This study showed that this new protection mechanism decreased ROS release, showing a lower superoxide anion production, and thus a lower injury. These findings were supported by Sun et al. [58] in experiments on isolated cardiomyocytes subjected to hypoxia/reoxygenation. For several years, our laboratory has been studying the cardioprotection mechanisms against I/R injury [61–66]. In previous papers we showed that A1 adenosine receptors and K^+_{ATP} sensitive channels were involved in PostC cardioprotection mechanism [65]. Later, other authors demonstrated that the decrease in ROS and $ONOO^-$[23], were involved in the protection conferred by PostC. We extended this concept and demonstrated that ischemic PostC attenuates the MMP-2[24] activation during the first minutes of reperfusion and the administration of doxycycline reproduces the PostC effect on the infarct size by inhibiting MMP-2 [66]. This way, and according to the aforementioned, PC and PostC shares the intracellular signaling mechanisms and exerts cardioprotection, decreasing the oxidative stress that occurs in reperfusion [67]. However, since the intervention takes place after the ischemic event occurs, the study of this endogenous protection mechanism is more interesting from the clinical viewpoint.

At least to our knowledge, we were the first to suggest a role for Trx-1 in PostC [68]. In isolated and perfused mice heart, we demonstrated that PostC decreases the infarct size in young mice, as we expected, and this cardioprotection was abolished in middle-aged and old mice. The reduction of infarct size in PostC is in concordance with Trx-1 levels preservation and with an improvement in the GSH/GSSG ratio (oxidative stress) in comparison to the I/R control group. Also, this cardioprotection was accompanied by the Akt activation and the phosphorylation and inhibition of GSK3β[25], both proteins related to cell survival pathway [68]. Therefore, our data suggests that the preservation of Trx-1 levels in young mice subjected to a PostC protocol would reduce oxidative stress and allow the RISK signaling pathway (Akt- GSK3β) activation to increase cell survival (Fig. 1).

[18] Diacylglycerol.
[19] Inosine 3-phosphate.
[20] Protein kinase C.
[21] Phosphatidyl inositol 3 kinase.
[22] Mitogen regulated kinase.
[23] Peroxynitrite.
[24] Matrix metalloproteinase-2.
[25] Glycogen Synthase Kinase 3 Beta.

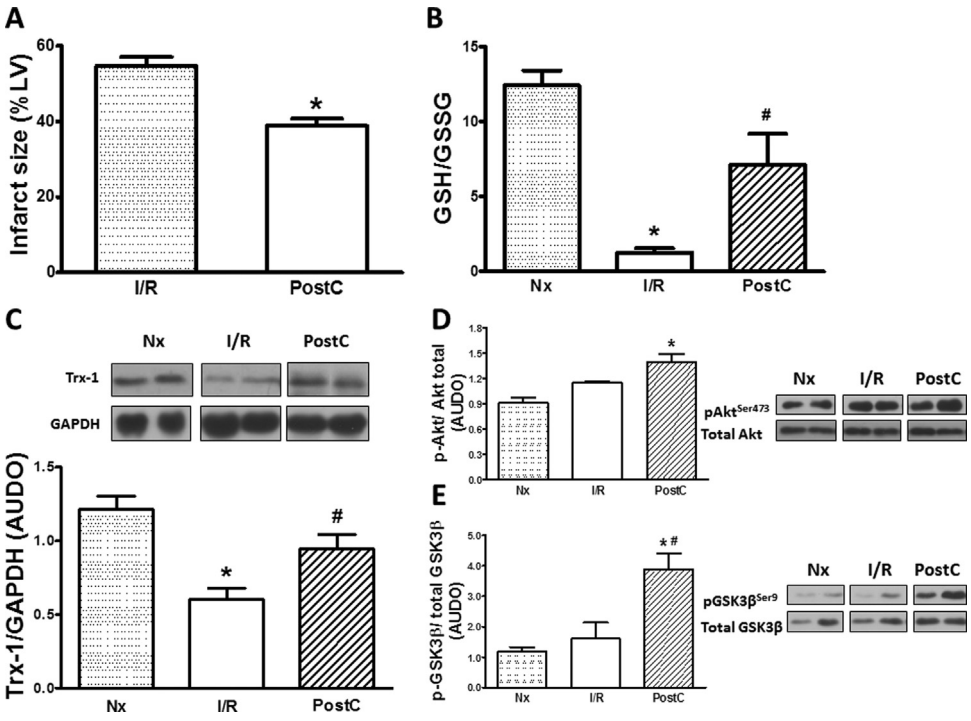

Fig. 1. Cardioprotection conferred by ischemic postconditiong involved thioredoxin-1. Panel A: Infarct size expressed as a percentage of the total left ventricular area. Infarct size decreased significantly in postconditioning (PostC) group. *p < 0.05 vs. I/R (ischemia/reperfusion protocol). Panel B: Oxidative stress. The GSH/GSSG ratio shows a significant increase in oxidative stress in I/R-Y group, the levels of GSH were decreased and levels of GSSG were increased compared to normoxic (Nx) condition. This disbalancein redox homeostasis returned to preischemic values after PostC protocol. *p < 0.05 vs. Nx; #p < 0.05 vs. I/R. Panel C: I/R decreases Trx-1 levels in young animals, and PostC preserves the protein expression levels. *p < 0.05 vs. Nx; #p < 0.05 vs. I/R. Panel D and E: Akt phosphorylation (Ser473) and GSK3β phosphorylation (Ser9) protein expression in normoxic (Nx), ischemia/reperfusion (I/R) and PostC young animals. PostC group shows a significant increase in Akt phosphorylation (Panel D) and GSK3β phosphorylation (Panel D). There were no significant changes in the cytosolic Akt and GSK3β protein expression. *p < 0.05 vs. Nx.

4. Thioredoxin in aging hearts

We have mentioned that Trx-1 may be involved in protective mechanisms against I/R, but all these studies have been performed in young and healthy individuals. However, it should be interesting to study Trx-1 behavior during aging. In aging there is a progressive loss in adaptability, and although many biological functions remain normal on a baseline state, when subjected to stress (as I/R), the loss of functional reserve is revealed [69]. It has been suggested that oxidative stress is exacerbated during the course of life in a way that exceeds the endogenous protection. In this regard, it is known that the aged hearts are more susceptible to I/R injury compared to young individuals, both in experimental models [70,71] and patients [72]. There is an increased oxidative damage, including modifications by protein oxidation [73], as well as damage mediated by calcium overload [74]. Moreover, the increase of the superoxide anion can react directly with the NO to form $ONOO^-$ and this compound increases with age [75]. From these findings it can be speculated that the protein expression or the activity of the endothelial cell antioxidant enzymes are reduced. Also, it has been demonstrated that the apoptosis of endothelial cells correlates with *in vitro* and *in vivo* aging [76,77] and that the induction of apoptosis is increased by TNF in elderly individual's endothelial cells, but it is completely blocked by Trx-1 over-expression in these cells. These data suggest that Trx-1 may actually improve endothelial function and rescue endothelial cells from age induced disorders. Therefore, in the heart it would be expected that an increased Trx-1 attenuates structural and functional changes produced by increased of age. In addition, Liu et al. [78] working with 19 and 4 month old F344BN rats subjected to 30 min of regional ischemia and 4 h of reperfusion, found an increased apoptosis in adult rat myocytes compared with young ones. Consistent with this research, Liu et al. [79] hypothesized, in another study but with the same models and the same groups, that the increased vulnerability of aged myocardial tissue subjected to I/R could be due to decreased antioxidant capacity, rather than to increased oxidant production after an episode of I/R.

Regarding cardioprotection, it is known that certain comorbidities, such as age, interfere in the cardioprotection mechanisms commonly used to reduce the infarct size in hearts subjected to I/R [80–82]. This way, an increased sensitivity of aged myocardium to oxidative stress results in the accumulation of cell and tissue damage, which may explain the high mortality rate after an episode of myocardial I/R in aging [83,84]. It has been demonstrated that aging is not only associated with increased susceptibility against ischemia, but also to a decreased recovery of myocardial function following ischemia [85]. This concept suggests that aging decreases the intrinsic tolerance to ischemia. This loss of tolerance to myocardial ischemia in mice starts during the middle-aged of life (12 month old) and it becomes more manifest during aging (18 month old and 24–28 month old) [86]. In this sense, Zhang et al. [87] demonstrated that the activity of Trx was significantly reduced in aging hearts even before they were subjected to myocardial I/R. For these reasons, infarct size was larger in aged C57/BI6 mice (20 month old). In a similar manner, Azhar et al. [88] demonstrated an increase in the infarct size in C57/BI6 mice at 22–24 month old, subjected to 45 min of ischemia and 4 h of reperfusion. However, this reduction of tolerance to ischemia was not evidenced in the infarct size of C57/BI6 mice at 13 month old, subjected to 30 min of ischemia and 2 h of reperfusion (*in vitro*) [89]. Taken together, and although it is clear that I/R injury is exacerbated in elderly populations, it is not clear whether this also occurs in middle-aged, when the deleterious effects of aging are already taking place [90,91]. This lack of studies in middle-aged is striking, since an ischemic episode

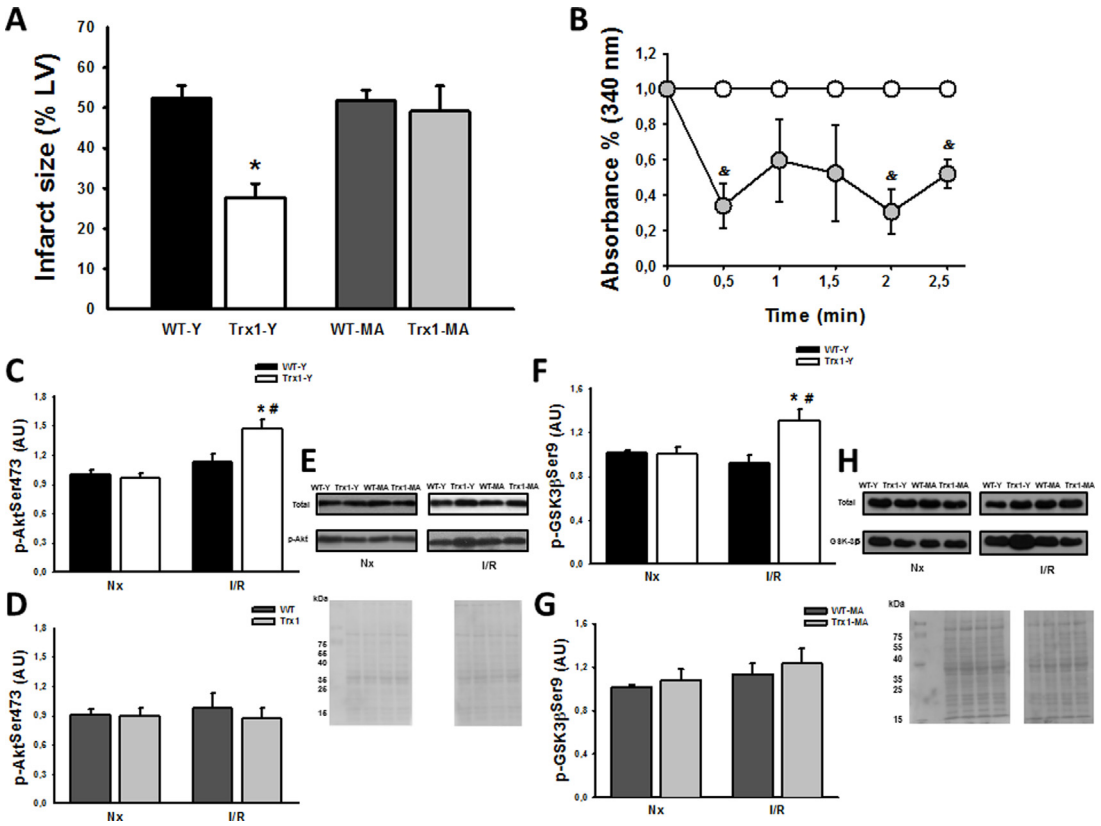

Fig. 2. Panel A shows that Trx-1 overexpression only reduced infarct size in young mice and in middle-aged mice this cardioprotective effect was abolished. Panel B shows that activity of Trx-1 decreased in middle-aged mice compared with young transgenic mice. Finally, Akt phosphorylation (PanelC) and GSK3β phosphorylation (Panel F) increased in Trx-1 young mice. There were not observe significant changes neither in Akt (Panel D) nor GSK3β phosphorylation (Panel G). Panel E and H show representative blots of total and phosphorylated Akt and GSK3-β, respectively.*$p < 0.05$ vs. wild type young mice (Wt-Y).

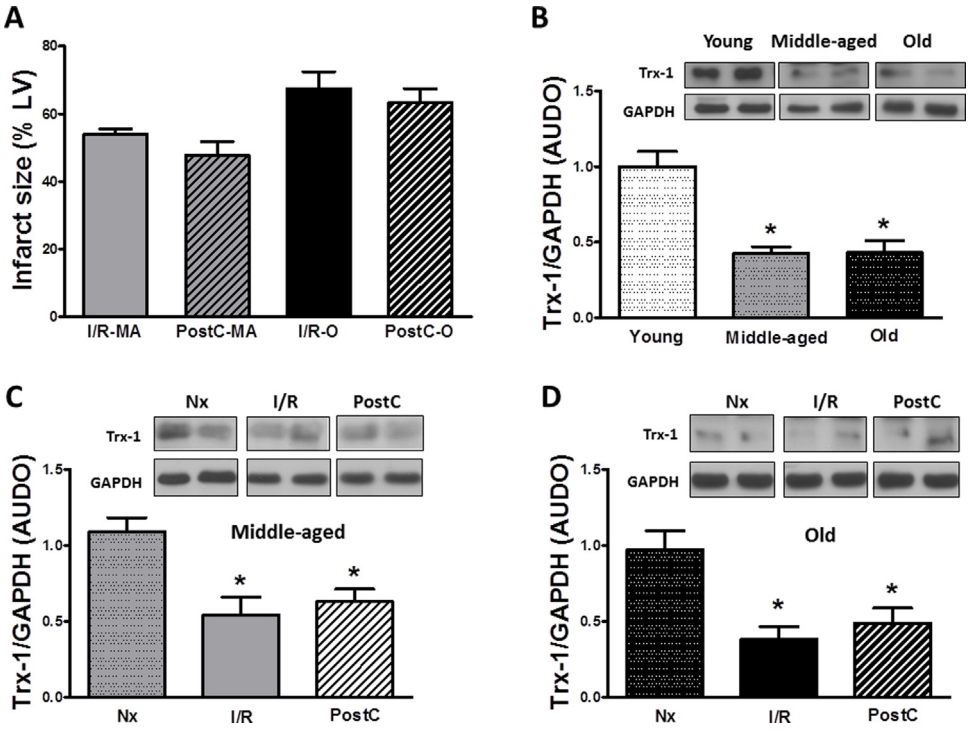

Fig. 3. Panel A: Infarct size expressed as a percentage of the total left ventricular area. PostC cardioprotective effect was abolished in middle-aged and old groups. Panel B: Trx-1 expression in normoxic (Nx) condition among young, middle-aged and old mice. In middle-aged and old mice decreases Trx-1 expression compared with young animals. *$p < 0.05$ vs. young mice. Panel C: Trx-1 expression in middle-aged animals in Nx, ischemia/reperfusion (I/R) and postconditioning (PostC) protocols. There were not significantly changes in Trx-1 expression in middle-aged group between I/R and PostC protocols. Panel D: Trx-1 expression in old animals in Nx, I/R and PostC protocols. There were not significantly changes in Trx-1 expression in old group between I/R and PostC protocols.

in patients begins at that stage of life, and they are not exclusive of advanced age [90,91]. However, it is known that even though oxidation processes start when life begins; it is in middle-aged that they reach sufficient levels to trigger deleterious mechanisms on different cell components [92], and this ROS increase is able to modify expression and/or activity of several proteins [93–95]. In a recent paper from our lab, we showed that young mice overexpressing Trx-1 have a smaller infarct size compared to their corresponding Wt mice [96]. However, this infarct size reduction was observed neither in Trx-1 middle-aged nor in young and middle-aged DN-Trx-1 mice. Although we found a significant increase in Trx-1 levels in young and middle-aged transgenic mice, Trx-1 activity was less in middle-aged mice and these findings were accompanied by an increase in the nitration of this protein. Finally, we also demonstrated that phosphorylation of Akt and GSK3β was increased only in young Trx-1 mice without any change in middle-aged mice and DN-Trx-1 mice (Fig. 2).

Our findings evidenced an increase in the infarct size only in old mice (20 month old), but we did not observe changes in the infarct size in middle-aged mice (12 month old). In this study we also demonstrated that PostC protocol did not confer protection in the middle-aged (12 month old) and in the old mice (>18 month old) [68]. These data are according with our finding that showed that Trx-1 levels in normoxic conditions, decreased in middle-age and old mice, compared with young mice (Fig. 3). These results show that the protection conferred by PostC was abolished in middle-aged and old animals, suggesting that the changes that can occur with aging are able to modify the behavior of the endogenous antioxidant systems, and therefore avoiding cardioprotection mechanism such as PC and PostC.

5. Conclusion

A better understanding of endogenous defense system against I/R injury, could prevent and reduce complications of the ischemic heart disease. Furthermore, the study of cardioprotection mechanisms (PC and PostC) in models with comorbidities such as age, is clearly fundamental for the potential extrapolation to the clinical setting. In this review we summarize data showing that the cardioprotective effect of Trx-1 *per se*, PC and PostC were abolished in middle age and old animals, at least in part by inactivation of the Trx system. Therefore, the lack of success of antioxidants therapies to treat ischemic heart disease could be solved avoiding the damage of Trx system. Further basic and clinical research are necessary to better understand the mechanisms involved in the inactivation of the antioxidants in order to prevent the oxidative stress deleterious effect, especially in ischemia and reperfusion injury and aging as well as in other comorbidities. This way the cardioprotective mechanisms can be useful and be applied in the clinical setting.

Conflict of interest

None to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phrs.2016.03.009.

References

[1] T. Ago, J. Sadoshima, Thioredoxin and ventricular remodeling, J. Mol. Cell Cardiol. 41 (2006) 762–773.
[2] H. Nakamura, Y. Hoshino, Y. Okuyama, J. Yodoi, Thioredoxin 1 as a new therapeutics, Adv. Drug Rev. 61 (4) (2009) 303–309.
[3] T.C. Laurent, E.C. Moore, P. Reichard, Enzymatic synthesis of deoxyribonucleotides IV. Isolation and characterization of thioredoxin, the hydrogen donor from *Escherichia coli* B, J. Biol. Chem. 239 (1964) 3436–3444.
[4] A. Holmgren, B.O. Söderberg, H. Eklund, C.I. Brändén, Three-dimensional structure of Escherichia coli thioredoxin-S2 to 28 Å resolution, U. S. A. Proc. Natl. Acad. Sci. 72 (6) (1975) 2305–2309.
[5] Jean-Francois Collet and, Joris messens structure, function, and mechanism of thioredoxin proteins, Antioxid. Redox Signal. 13 (2010) 1205–1216.
[6] K. Shioji, C. Kishimoto, H. Nakamura, H. Masutani, Z. Yuan, S. Oka, J. Yodoi, Overexpression of thioredoxin-1 in transgenic mice attenuates adriamycin-induced cardiotoxicity, Circulation 106 (11) (2002) 1403–1409.
[7] T. Rassaf, P. Luedike, Between nitros(yl) ation and nitration: regulation of thioredoxin-1 in myocardial ischemia/reperfusion injury, J. Mol. Cell Cardiol. 49 (3) (2010) 343–346.
[8] T. Turoczi, V.W. Chang, R.M. Engelman, N. Maulik, Y.S. Ho, D.K. Das, Thioredoxin redox signaling in the ischemic heart: an insight with transgenic mice overexpressing Trx1, J. Mol. Cell Cardiol. 35 (6) (2003) 695–704.
[9] S. Matsushima, D. Zablocki, J. Sadoshima, Application of recombinant thioredoxin1 for treatment of heart desease, J. Mol. Cell Cardiol. 51 (4) (2011) 570–573.
[10] A. Mitsui, J. Hamuro, H. Nakamura, N. Kondo, Y. Hirabayashi, S. Ishizaki-Koizumi, T. Hirakawa, T. Inoue, J. Yodoi, Overexpression of human thioredoxin in transgenic mice controls oxidative stress and life span, Antioxid. Redox Signal. 4 (2002) 693–696.
[11] S. Kaga, L. Zhan, M. Matsumoto, N. Maulik, Resveratrol enhances neovascularization in the infarcted rat myocardium through the induction of thioredoxin-1: heme oxygenase-1 and vascular endothelial growth factor, J. Mol. Cell Cardiol. 39 (5) (2005) 813–822.
[12] J. Altschmied, J. Haendeler, Thioredoxin-1 and endothelial cell aging: role in cardiovascular diseases, Antioxid. Redox Signal. 11 (2009) 1733–1740.
[13] M. Aota, K. Matsuda, N. Isowa, H. Wada, J. Yodoi, T. Ban, Protection against reperfusion-induced arrhythmias by human thioredoxin, J. Cardiovasc. Pharmacol. 27 (5) (1996) 727–732.
[14] H. Nakamura, J. Vaage, G. Valen, C.A. Padilla, M. Björnstedt, A. Holmgren, Measurements of plasma glutaredoxin and thioredoxin in healthy volunteers and during open-heart surgery, Free Radic. Biol. Med. 24 (7-8) (1998) 1176–1186.
[15] M. Kihlström, Protection effect of endurance training against reoxygenation-induced injuries in rat heart, J. Appl. Physiol. 68 (4) (1990) 1672–1678.
[16] L. Tao, E. Gao, N.S. Bryan, Y. Qu, H.R. Liu, A. Hu, T.A. Christopher, B.L. Lopez, J. Yodoi, W.J. Koch, M. Feelisch, X.L. Ma, Cardioprotective effects of thioredoxin in myocardial ischemia and reperfusion: role of S-nitrosation, Proc. Natl. Acad. Sci. U. S. A. 101 (31) (2004) 11471–11476.
[17] E.J. Meuillet, D. Mahadevan, M. Berggren, A. Coon, G. Powis, Thioredoxin-1 binds to the C2 domain of PTEN inhibiting PTEN's lipid phosphatase activity and membrane binding: a mechanism for the functional loss of PTEN's tumor suppressor activity, Arch. Biochem. Biophys. 429 (2) (2004) 123–133.
[18] M.K. Saini, S.N. Sanyal, PTEN regulates apoptotic cell death through PI3-K/Akt/GSK3β signaling pathway in DMH induced early colon carcinogenesis in rat, Exp. Mol. Pathol. 93 (1) (2012) 135–146.
[19] H.L. Ha, D.Y. Yu, HBx-induced reactive oxygen species activates hepatocellular carcinogenesis via dysregulation of PTEN/Akt pathway, World J. Gastroenterol. 16 (39) (2010) 4932–4937.
[20] L. Zu, X. Zheng, B. Wang, N. Parajuli, C. Steenbergen, L.C. Becker, Z.P. Cai, Ischemic preconditioning attenuates mitochondrial localization of PTEN induced by ischemia-reperfusion, Am. J. Physiol. Heart Circ. Physiol. 300 (6) (2011) 2177–2186.
[21] D.M. Flaherty, M.M. Monick, S.L. Hinde, Human alveolar macrophages are deficient in PTEN, J. Biol. Chem. 281 (8) (2006) 5058–5064.
[22] D.J. Hausenloy, D.M. Yellon, Reperfusion injury salvage kinase signalling: taking a RISK for cardioprotection, Heart Fail. Rev. 12 (3-4) (2007) 217–234.
[23] C. Penna, M.G. Perrelli, P. Pagliaro, Mitochondrial pathways, permeability transition pore, and redox signaling in cardioprotection: therapeutic implications, Antioxid. Redox Signal. 18 (5) (2013) 556–599.
[24] S. Sanada, I. Komuro, M. Kitakaze, Pathophysiology of myocardial reperfusion injury: preconditioning, postconditioning, and translational aspects of protective measures, Am. J. Physiol. Heart Circ. Physiol. 301 (5) (2011) H1723–H1741.
[25] H. Ge, M. Zhao, S. Lee, Z. Xu, Mitochondrial Src tyrosine kinase plays a role in the cardioprotective effect of ischemic preconditioning by modulating complex I activity and mitochondrial ROS generation, Free Radic. Res. 49 (10) (2015) 1210–1217.
[26] P. Yu, J. Zhang, S. Yu, Z. Luo, F. Hua, L. Yuan, Z. Zhou, Q. Liu, X. Du, S. Chen, L. Zhang, G. Xu, Protective effect of sevoflurane postconditioning against cardiac Ischemia/Reperfusion injury via ameliorating mitochondrial impairment oxidative stress and rescuing autophagic clearance, PLoS One 10 (8) (2015) e0134666.

[27] C.E. Murry, R. Jennings, K.A. Reimer, Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium, Circulation 74 (1986) 1124–1136.
[28] A. Cave, G. Horowitz, C. Apstein, Can ischemic preconditioning protect against hypoxia-induced damage? Studies of contractile function in isolated rat hearts, J. Mol. Cell Cardiol. 26 (1994) 1471–1486.
[29] K. Ytrehus, Y. Liu, J. Downey, Preconditioning protects ischemic rabbit heart by protein kinase C activation, Am. J. Physiol. 266 (1994) H1145–H1152.
[30] G.S. Liu, J. Thornton, D.M. Van Winkle, A.W. Stanley, R.A. Olsson, J.M. Downey, Protection against infarction afforded by preconditioning is mediated by A1 adenosine receptors in rabbit heart, Circulation 84 (1991) 350–356.
[31] M. Leesar, M. Stoddard, M. Ahmed, J. Broadbent, R. Bolli, Preconditioning of human miocardium with adenosine during coronary angioplasty, Circulation 95 (1997) 2500–2507.
[32] V.J. McIntosh, R.D. Lasley, Adenosine receptor-mediated cardioprotection: are all 4 subtypes required or redundant? J. Cardiovasc. Pharmacol. Ther. 17 (1) (2012) 21–33.
[33] A. Banerjee, C. Locke-Winter, K.B. Rogers, M.B. Mitchell, E.C. Brew, C.B. Cairns, D.D. Bensard, A.H. Harken, Preconditioning against myocardial dysfunction after ischemia and reperfusion by an alpha 1-adrenergic mechanism, Circ. Res. 73 (1993) 656–670.
[34] O. Oldenburg, Q. Qin, A.R. Sharma, M.V. Cohen, J.M. Downey, J.N. Benoit, Acetylcholine leads to free radical production dependent on K(ATP) channels, G(i) proteins, phosphatidylinositol 3-kinase and tyrosine kinase, Cardiovasc. Res. 55 (2002) 544–552.
[35] T. Krieg, L. Cui, Q. Qin, M.V. Cohen, J.M. Downey, R.O.S. Mitochondrial, generation following acetylcholine-induced EGF receptor transactivation requires metalloproteinase cleavage of proHB-EGF, J. Mol. Cell Cardiol. 36 (2004) 435–443.
[36] C.S. Carr, R.J. Hill, H. Masamune, S.P. Kennedy, D.R. Knight, W.R. Tracey, D.M. Yellon, Evidence for a role for both the adenosine A1 and A3 receptors in protection of isolated human atrial muscle against simulated ischaemia, Cardiovasc. Res. 36 (1997) 52–59.
[37] H. Takano, R. Bolli, R.G. Black Jr., E. Kodani, X.L. Tang, Z. Yang, S. Bhattacharya, J.A. Auchampach, A(1) or A(3) adenosine receptors induce late preconditioning against infarction in conscious rabbits by different mechanisms, Circ. Res. 88 (2001) 520–528.
[38] M.V. Cohen, C.P. Baines, J.M. Downey, Ischemic preconditioning: from adenosine receptor to KATP channel, Annu. Rev. Physiol. 10 (2000) 62–79.
[39] C. Page, A.F. Doubell, Mitogen-activated protein kinase (MAPK) in cardiac tissues, Mol. Cell Biochem. 157 (1996) 12–26.
[40] Y. Wang, Mitogen-activated protein kinases in heart development and diseases, Circulation 116 (2007) 1413–1423.
[41] C.P. Baines, M.V. Cohen, J.M. Downey, Signal transduction in ischemic preconditioning: the role of kinases and mitochondrial K(ATP) channels, J. Cardiovasc. Electrophysiol. 10 (1999) 741–754.
[42] N. Maulik, M. Watanabe, Y.L. Zu, C.K. Huang, G.A. Cordis, J.A. Schley, D.K. Das, Ischemic preconditioning triggers the activation of MAP kinases and MAPKAP kinase 2 in rat hearts, FEBS Lett. 396 (1996) 233–237.
[43] N. Maulik, M. Sato, B.D. Price, D.K. Das, An essential role of NFkappaB in tyrosine kinase signaling of p38 MAP kinase regulation of myocardial adaptation to ischemia, FEBS Lett. 429 (3) (1998) 365–369.
[44] Z. Yao, J. Tong, X. Tan, C. Li, Z. Shao, W.C. Kim, T.L. vanden Hoek, L.B. Becker, C.A. Head, P.T. Schumacker, Role of reactive oxygen species in acetylcholine-induced preconditioning in cardiomyocytes, Am. J. Physiol. 277 (1999) H2504–2509.
[45] O. Oldenburg, S.D. Critz, M.V. Cohen, J.M. Downey, Acetylcholine-induced production of reactive oxygen species in adult rabbit ventricular myocytes is dependent on phosphatidylinositol 3- and Src-kinase activation and mitochondrial K (ATP) channel opening, J. Mol. Cell Cardiol. 35 (2003) 653–660.
[46] L.B. Valdez, T. Zaobornyj, S. Bombicino, D.E. Iglesias, A. Boveris, M. Donato, V. D'Annunzio, B. Buchholz, R.J. Gelpi, I. Complex, syndrome in myocardial stunning and the effect of adenosine, Free Radic. Biol. Med. 51 (6) (2011) 1203–1212.
[47] L.B. Becker, New concepts in reactive oxygen species and cardiovascular reperfusion physiology, Cardiovasc. Res. 15 (3) (2004) 461–470.
[48] M.L. Hess, R.C. Kukreja, Free radicals, calcium homeostasis, heat shock proteins, and myocardial stunning, Ann. Thorac. Surg. 60 (3) (1995) 760–766.
[49] H. Nakamura, K. Nakamura, J. Yodoi, Redox regulation of cellular activation, Annu. Rev. Immunol. 15 (1997) 351–369.
[50] C.C. Chiueh, T. Andoh, P.B. Chock, Induction of thioredoxin and mitochondrial survival proteins mediates preconditioning-induced cardioprotection and neuroprotection, Ann. N. Y. Acad. Sci. 1042 (2005) 403–418.
[51] T. Ago, I. Yeh, M. Yamamoto, M. Schinke-Braun, J.A. Brown, B. Tian, J. Sadoshima, Thioredoxin1 upregulates mitochondrial proteins related to oxidative phosphorylation and TCA cycle in the heart, Antioxid. Redox Signal. 8 (9–10) (2006) 1635–1650.
[52] D.K. Das, N. Maulik, Preconditioning potentiates redox signaling and converts death signal into survival signal, Arch. Biochem. Biophys. 420 (2) (2003) 305–311.
[53] Z.Q. Zhao, J.S. Corvera, M.E. Halkos, F. Kerendi, N.P. Wang, R.A. Guyton, J. Vinten-Johansen, Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning, Am. J. Physiol. Heart Circ. Physiol. 285 (2003) H579–H588.
[54] A. Tsang, D.J. Hausenloy, M.M. Mocanu, Yellon D.M. Postconditioning, a form of modified reperfusion protects the myocardium by activating the phosphatidylinositol 3-kinase-Akt pathway, Circ. Res. 95 (2004) 230–232.
[55] X.M. Yang, J.B. Proctor, L. Cui, T. Krieg, J.M. Downey, M.V. Cohen, Multiple, Brief coronary occlusions during early reperfusion protect rabbit hearts by targeting cell signaling pathways, J. Am. Coll. Cardiol. 44 (2004) 1103–1110.
[56] G. Gritsopoulos, E.K. Iliodromitis, A. Zoga, D. Farmakis, E. Demerouti, A. Papalois, I.A. Paraskevaidis, D.T. Kremastinos, Remote postconditioning is more potent than classic postconditioning in reducing the infarct size in anesthetized rabbits, Cardiovasc. Drugs Ther. 23 (3) (2009) 193–198.
[57] D. Weihrauch, J.G. Krolikowski, M. Bienengraeber, J.R. Kersten, D.C. Warltier, Pagel Morphine enhances isoflurane-induced postconditioning against myocardial infarction: the role of phosphatidylinositol-3-kinase and opioid receptors in rabbits, Anesth. Analg. 101 (2005) 942–949.
[58] H.Y. Sun, N.P. Wang, F. Kerendi, M. Halkos, H. Kin, R.A. Guyton, J. Vinten-Johansen, Z.Q. Zhao, Hypoxic postconditioning reduces cardiomyocyte loss by inhibiting ROS generation and intracellular Ca2+ overload, Am. J. Physiol. Heart Circ. Physiol. 288 (2005) H1900–H1908.
[59] C.E. Darling, P.B. Solari, C.S. Smith, M.I. Furman, K. Przyklenk, Postconditioning' the human heart: multiple balloon inflations during primary angioplasty may confer cardioprotection, Basic Res. Cardiol. 102 (2007) 274–278.
[60] H. Kin, Z.Q. Zhao, H.Y. Sun, N.P. Wang, J.S. Corvera, M.E. Halkos, F. Kerendi, R.A. Guyton, J. Vinten-Johansen, Postconditioning attenuates myocardial ischemia-reperfusion injury by inhibiting events in the early minutes of reperfusion, Cardiovasc. Res. 62 (2004) 74–85.
[61] V. D'Annunzio, M. Donato, L. Erni, V. Miksztowicz, B. Buchholz, C.L. Carrión, L. Schreier, R. Wikinski, R.J. Gelpi, G. Berg, N. Basso, Rosuvastatin given during reperfusion decreases infarct size and inhibits matrix metalloproteinase-2 activity in normocholesterolemic and hypercholesterolemic rabbits, J. Cardiovasc. Pharmacol. 53 (2) (2009) 137–144.
[62] M. Donato, V. D'Annunzio, B. Buchholz, V. Miksztowicz, C.L. Carrión, L.B. Valdez, T. Zaobornyj, L. Schreier, R. Wikinski, A. Boveris, G. Berg, R.J. Gelpi, Role of matrix metalloproteinase-2 in the cardioprotective effect of ischaemic postconditioning, Exp. Physiol. 95 (2) (2010) 274–281.
[63] V. D'Annunzio, M. Donato, B. Buchholz, V. Pérez, V. Miksztowicz, G. Berg, R.J. Gelpi, High cholesterol diet effects on ischemia-reperfusion injury of the heart, Can. J. Physiol. Pharmacol. 90 (9) (2012) 1185–1196.
[64] B. Buchholz, V. DíAnnunzio, J.F. Giani, N. Siachoque, F.P. Dominici, D. Turyn, V. Perez, M. Donato, R.J. Gelpi, Ischemic postconditioning reduces infarct size through the α1-Adrenergic receptor pathway, J. Cardiovasc. Pharmacol. 63 (2014) 504–511.
[65] M. Donato, V. D'Annunzio, G. Berg, G. Gonzalez, L. Schreier, C. Morales, R.L. Wikinski, R.J. Gelpi, Ischemic postconditioning reduces infarct size by activation of A1 receptors and K+(ATP) channels in both normal and hypercholesterolemic rabbits, J. Cardiovasc. Pharmacol. 49 (5) (2007) 287–292.
[66] B. Buchholz, V. Perez, N. Siachoque, V. Miksztowicz, G. Berg, M. Rodríguez, M. Donato, R.J. Gelpi, Dystrophin proteolysis: a potential target for MMP-2 and its prevention by ischemic preconditioning, Am. J. Physiol. Heart Circ. Physiol. 307 (1) (2014) H88–96.
[67] A. Skyschally, R. Schulz, G. Heusch, Pathophysiology of myocardial infarction: protection by ischemic pre- and postconditioning, Herz 33 (2) (2008) 88–100.
[68] V. Perez, V. DÁnnunzio, T. Mazo, T. Marchini, L. Cáceres, P. Evelson, R.J. Gelpi, Ischemic postconditioning confers cardioprotection and prevents reduction of Trx-1 in young mice, but not in middle-aged and old mice, Mol. Cell Biochem. (2016), http://dx.doi.org/10.1007/s11010-016-2677-2.
[69] B.J. Cusack, P.S. Mushlin, T. Andrejuk, L.D. Voulelis, R.D. Olson, Aging alters the force-frequency relationship and toxicity of oxidative stress in rabbit heart, Life Sci. 48 (1991) 1769–1777.
[70] V.V. Frolkis, R.A. Frolkis, L.S. Mkhitarian, V.E. Fraifeld, Age-dependent effects of ischemia and reperfusion on cardiac function and Ca2+ transport in myocardium, Gerontology 37 (1991) 233–239.
[71] E.J. Lesnefsky, D.S. Gallo, J. Ye, T.S. Whittingham, W.D. Lust, Aging increases ischemia-reperfusion injury in the isolated, buffer perfused heart, J. Lab. Clin. Med. 124 (1994) 843–851.
[72] E.J. Lesnefsky, C.F. Lundergan, J.M. Hodgson, R. Nair, J.S. Reiner, S.W. Greenhouse, R.M. Califf, A.M. Ross, Increased left ventricular dysfunction in elderly patients despite successful thrombolysis: the GUSTO-I angiographic experience, J. Am. Coll. Cardiol. 28 (1996) 331–337.
[73] W.D. Flitter, Free radicals and myocardial reperfusion injury, Br. Med. Bull. 49 (1993) 545–555.
[74] K. Atake, D. Chen, S. Levitsky, E. Jimenez, H. Feinberg, Effect of aging on intracellular Ca2+, pHi, and contractility during ischemia and reperfusion, Circulation 86 (1992) 371–376.
[75] B. Van der Loo, R. Labugger, J.N. Skepper, M. Bachschmid, J. Kilo, J.M. Powell, M. Palacios-Callender, J.D. Etrusalimsky, T. Quashning, T. Malinski, D. Gygi, V. Ullrich, T.F. Luscher, Enhanced peroxynitrite formation is associated with vascular aging, J. Exp. Med. 192 (2000) 1731–1744.
[76] K. Asai, R.K. Kudej, Y.T. Shen, G.P. Yang, G. Takagi, A.B. Kidej, Y.J. Geng, N. Sato, J.B. Nazareno, D.E. Vatner, F. Natividad, S.P. Bishop, S.F. Vatner, Peripheral vascular endothelial dysfunction and apoptosis in old monkeys, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 1493–1499.
[77] J. Hoffmann, J. Haendeler, A. Aicher, L. Rossig, M. Vasa, A.M. Zeiher, S. Dimmeler, Aging enhances the sensitivity of endothelial cells toward apoptotic stimuli: important role of nitric oxide, Circ. Res. 89 (2001) 709–715.

[78] P. Liu, B. Xu, T.A. Cavalieri, C.E. Hock, Age-related difference in myocardial function and inflammation in a rat model of myocardial ischemia-reperfusion, Cardiovasc. Res. 56 (2002) 443–453.
[79] P. Liu, B. Xu, T.A. Cavalieri, C.E. Hock, Attenuation of antioxidative capacity enhances reperfusion injury in aged rat myocardium after MI/R, Am. J. Physiol. Heart Circ. Physiol. 287 (2004) 2719–2727.
[80] P. Ferdinandy, D.J. Hausenloy, G. Heusch, G.F. Baxter, R. Schulz, Interaction of risk factors, comorbidities, and comedications with ischemia/reperfusion injury and cardioprotection by preconditioning, postconditioning, and remote conditioning, Pharmacol. Rev. 66 (4) (2014) 1142–1174.
[81] K. Przyklenk, Efficacy of cardioprotective 'conditioning' strategies in aging and diabetic cohorts: theco-morbidity conundrum, Drugs Aging 28 (5) (2011) 331–343.
[82] K. Boengler, R. Schulz, G. Heusch, Loss of cardioprotection with ageing, Cardiovasc. Res. 83 (2) (2009) 247–261.
[83] P. Abete, C. Napoli, G. Santoro, N. Ferrara, I. Tritto, M. Chiariello, F. Renco, G. Ambrosio, Age-related decrease in cardiac tolerance to oxidative stress, J. Mol. Cell Cardiol. 31 (1999) 227–236.
[84] R.A. Fenton, E.W. Dickson, T.E. Meyer, J.G. Dobson, Aging reduces the cardioprotective effect of ischemic preconditioning in the rat heart, J. Mol. Cell Cardiol. 32 (2000) 1371–1375.
[85] M.S. Sumeray, D.M. Yellon, Characterisation and validation of a murine model of global ischaemiareperfusion injury, Mol. Cell Biochem. 186 (1998) 61–68.
[86] L. Willems, A. Zatta, K. Holgren, K.J. Ashton, J.P. Headrick, Age-related changes in ischemic tolerance in male and female mouse hearts, J. Mol. Cell Cardiol. 38 (2) (2005) 245–256.
[87] H. Zhang, L. Tao, X. Jiao, E. Gao, B.L. Lopez, T.A. Christopher, W. Koch, X.L. Ma, Nitrative thioredoxin inactivation as a cause of enhanced myocardial ischemia/reperfusion injury in the aging heart, Free Radic. Biol. Med. 43 (2007) 39–47.
[88] G. Azhar, W. Gao, L. Liu, J.Y. Wei, Ischemia-reperfusion in the adult mouse heart influence of age, Exp. Gerontol. 34 (1999) 699–714.
[89] K. Boengler, I. Konietzka, A. Buechert, Y. Heinen, D. Garcia-Dorado, G. Heusch, R. Schulz, Loss of ischemic preconditioning's cardioprotection in aged mouse hearts is associated with reduced gap junctional and mitochondrial levels of connexin 43, Am. J. Physiol. Heart Circ. Physiol. 292 (4) (2007) H1764–9.
[90] G.S. Uhl, P.W. Farrell, Myocardial infarction in young adults: risk factors and natural history, Am. Heart J. 105 (1983) 548–553.
[91] D. Cohen, D.G. Manuel, P. Tugwell, T. Ramsay, C. Sanmartin, Inequity in primary and secondary preventive care for acute myocardial infarction? Use by socioeconomic status across middle-aged and older patients, Can. J. Cardiol. 29 (2013) 1579–1585.
[92] Q. Fan, M. Chen, X. Fang, W.B. Lau, L. Xue, L. Zhao, H. Zhang, Y.H. Liang, X. Bai, H.Y. Niu, J. Ye, Q. Chen, X. Yang, M. Liu, Aging might augment reactive oxygen species (ROS) formation and affect reactive nitrogen species (RNS) level after myocardial ischemia/reperfusion in both humans and rats, Age (Dordr.) 35 (2013) 1017–1026.
[93] E. Babušíková, J. Lehotský, D. Dobrota, P. Račay, P. Kaplán, Age-associated changes in Ca(2+)-ATPase and oxidative damage in sarcoplasmic reticulum of rat heart, Physiol. Res. 61 (2012) 453–460.
[94] K.B. Choksi, J.E. Nuss, J.H. DeFord, J. Papaconstantinou, Mitochondrial electron transport chain functions in longlived Ames dwarf mice, Aging (Milano) 3 (8) (2011) 754–767.
[95] J.Y. Kim, O.Y. Kim, J.K. Paik, D.Y. Kwon, H.J. Kim, J.H. Lee, Association of age-related changes in circulating intermediary lipid metabolites, inflammatory and oxidative stress markers, and arterial stiffness in middle-aged men, Age (Dordr.) 35 (2013) 1507–1519.
[96] V. DÁnnunzio, V. Perez, T. Mazo, B. Buchholz, M.C. Carreras, J.J. Poderoso, J. Sadoshima, R.J. Gelpi, Loss of myocardial protection against myocardial infarction in middle aged transgenic mice overexpressing cardiac thioredoxin-1, Oncotarget (2016), http://dx.doi.org/10.18632/oncotarget.7726.