Pharmacological Research 109 (2016) 24–31

Contents lists available at ScienceDirect

Pharmacological Research

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

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

Veronica D'Annunzio[a,b,1,3], Virginia Perez[a,b,2,3], Alberto Boveris[a,1], Ricardo J. Gelpi[a,b,*,1,4], Juan J. Poderoso[c,1,4]

[a] Institute of Biochemistry and Molecular Medicine (IBIMOL, UBA-CONICET), Argentina
[b] Institute of Cardiovascular Physiopathology, Department of Pathology, Faculty of Medicine, University of Buenos Aires, Argentina
[c] Laboratory of Oxygen Metabolism, University Hospital, University of Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 4 March 2016
Received in revised form 9 March 2016
Accepted 10 March 2016
Available online 14 March 2016

Keywords:
Heart
Ischemia/reperfusion
Thioredoxin
Preconditioning
Postconditioning
Aging

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

* Corresponding author at: Institute of Cardiovascular Physiopathology, Faculty of Medicine, University of Buenos Aires. JE Uriburu 950-2nd floor, Buenos Aires, 1114, Argentina.
E-mail addresses: rgelpi@fmed.uba.ar, ricardogelpi@fibertel.com.ar (R.J. Gelpi).
[1] Member of the National Council of Scientific and Technological Research (CONICET), Argentina.
[2] Doctoral fellowship of the National Council of Scientific and Technological Research (CONICET), Argentina.
[3] Both authors contributed equally to this manuscript.
[4] Both seniors authors contributed equally to this manuscript.
[1] Thioredoxin-1
[2] Cytidine diphosphate
[3] Thioredoxin reductase.
[4] Nicotinamine adenine dinucleotid phosphate.

http://dx.doi.org/10.1016/j.phrs.2016.03.009
1043-6618/

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.