

Thioredoxin A Key Regulator of Cardiovascular Homeostasis

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Abstract—The thioredoxin (TRX) system (TRX, TRX reductase, and NADPH) is a ubiquitous thiol oxidoreductase system that regulates cellular reduction/oxidation (redox) status. The oxidation mechanism for disease pathogenesis states that an imbalance in cell redox state alters function of multiple cell pathways. In this study, we review the essential role for TRX to limit oxidative stress directly via antioxidant effects and indirectly by protein-protein interaction with key signaling molecules, such as apoptosis signal-regulating kinase 1. We propose that TRX and its endogenous regulators are important future targets to develop clinical therapies for cardiovascular disorders associated with oxidative stress. (*Circ Res.* 2003;93:1029-1033.)

Key Words: antioxidants ■ cardiovascular diseases ■ endothelium ■ smooth muscle ■ signal transduction

Although the role of oxidative stress in cardiovascular disease is well established, the protective mechanisms of antioxidants remain poorly defined. Recent data show specific roles for thioredoxin (TRX) as a critical protective system via direct (antioxidant) and indirect (regulation of signal transduction) effects.

General Function of Thioredoxin (TRX) System

Regulation of Reduction/Oxidation (Redox) Status in Cell

The regulation of cellular redox balance is critically determined by the activity of several antioxidant systems. The ubiquitously expressed thiol-reducing systems include the TRX, glutaredoxin, and glutathione systems.^{1,2} The TRX system (TRX, TRX reductase, and NADPH) reduces oxidized cysteine groups on protein through an interaction with the redox-active center of TRX (Cys-Gly-Pro-Cys) to form a disulfide bond, which in turn can be reduced by TRX reductase and NADPH (Figure 1). TRX seems to exert most of its antioxidant (reactive oxygen species [ROS]-scavenging) properties through TRX peroxidase, which uses SH groups as reducing equivalents.³ TRX reduces the oxidized form of TRX peroxidase, and the reduced TRX peroxidase scavenges ROS, such as H₂O₂.⁴ A second TRX (TRX-2) was identified in mitochondria.^{5,6} TRX-2 has a conserved TRX catalytic site and a consensus signal sequence for mitochondrial translocation, but little is known regarding TRX-2 function. Unless indicated, TRX refers to the cytosolic TRX-1 in this review.

Activation of Transcription Factors (Nuclear Factor- κ B, Nuclear Receptors, and AP-1)

TRX selectively stimulates DNA-binding of transcription factors that are important for the cellular responses to oxidative stress, apoptosis, and tumorigenesis. TRX increases DNA binding of nuclear factor (NF)- κ B by reducing cysteine 62 of the NF- κ B p50 subunit.⁷ TRX also increases the expression of glucocorticoid receptor-responsive and estrogen receptor-responsive genes by associating with their DNA binding domains.^{8,9} TRX increases AP-1 activity indirectly via binding to another nuclear redox protein, redox factor 1 (Ref-1).¹⁰ Ref-1 associates transiently with AP-1 and reduces the conserved cysteines in Fos and Jun, thus enhancing their DNA binding activity.¹¹

Growth-Promoting Effects

TRX has many growth factor-like properties, including secretion, cell-surface binding, and catalytic activity.^{12,13} Because TRX mutated at the catalytic site (cysteines 32 and 35) cannot stimulate cell growth,¹³ the proliferative effect of TRX seems to be redox-dependent. Likely mechanisms include an increased supply of reducing equivalents for DNA synthesis and activation of transcription factors that regulate cell growth.¹⁴

Thioredoxin in Vascular Endothelium and Smooth Muscle

TRX is ubiquitously expressed in endothelial cells (ECs)¹⁵ and protects ECs from H₂O₂-induced cytotoxicity.¹⁶ Because treatment with H₂O₂ increased TRX expression in ECs, it seems that TRX is an oxidative stress-inducible protein. Of

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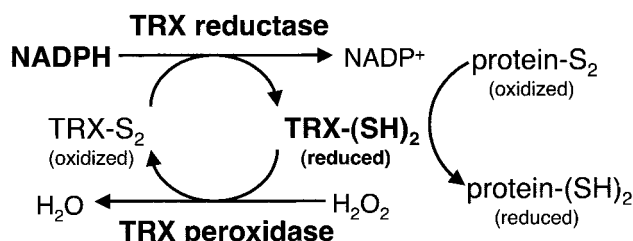


Figure 1. The TRX system.

interest, Ejima et al¹⁷ showed that estrogen-mediated protection against H₂O₂ required induction of TRX and TRX reductase. Das et al¹⁸ found in human lung microvascular ECs that exogenous TRX induced mitochondrial manganese superoxide dismutase (Mn SOD). Induction of Mn SOD by TRX was specific, because other antioxidant enzymes, including copper zinc SOD (Cu/Zn SOD) and catalase, were not induced. Thus, it is possible that the protective effects of TRX are mediated in part through Mn SOD induction. Finally, TRX may also have a role in protecting ECs from nitrosative stress, because overexpressing TRX prevented endothelial nitric oxide synthase (eNOS) degradation induced by excessive NO.¹⁹

TRX is also ubiquitously expressed in medial vascular smooth muscle cells (VSMCs) of normal arteries.¹⁵ Schulze et al²⁰ showed in human aortic VSMCs that adenoviral gene transfer of TRX enhanced TRX enzyme activity and significantly increased DNA synthesis, suggesting a role for TRX in VSMC proliferation. Importantly, expression of TRX in VSMCs is not regulated by ROS, unlike ECs, because no change in TRX expression was observed after treatment with H₂O₂ or platelet-derived growth factor (PDGF).²⁰ In rat aortic VSMCs, Wiesel et al²¹ showed that TRX contributed to lipopolysaccharide-induced and interleukin 1 β -induced heme oxygenase (HO)-1 expression mediated by increased AP-1 activity. Because the induction of HO-1 in response to cellular stress is believed to be an important antiapoptotic mechanism, HO-1 represents another TRX-protective mechanism.

Thioredoxin Expression in Cardiovascular Disease

In human coronary atherosclerotic specimens, TRX expression is enhanced throughout the vessel wall.¹⁵ The greatest increases were observed in ECs and infiltrating macrophages within the neointimal plaques.^{15,22} In balloon-injured rat carotid arteries, TRX expression increased in regenerating ECs.²² Takagi et al²² suggested that NO produced by inducible NOS (iNOS) plays a crucial role in induction of TRX, because the localization of iNOS strongly correlated with TRX. Because excess NO production by iNOS may be cytotoxic by forming peroxynitrite,²³ the findings suggest that induction of TRX represents a protective mechanism against nitrosative and oxidative stress.

Serum TRX levels are elevated in conditions associated with oxidative stress and inflammation, such as human immunodeficiency virus²⁴ and rheumatoid arthritis.²⁵ Kishimoto et al²⁶ reported that serum TRX levels were signifi-

cantly elevated in patients with acute coronary syndromes and dilated cardiomyopathy compared with control subjects. In addition, serum TRX levels correlated positively with the severity of New York Heart Association functional class and negatively with left ventricular ejection fraction. These results suggest a possible association between TRX secretion and the severity of heart failure. TRX is increased in both inflammatory cells and myocytes during myocarditis.²⁷ These results support the positive association between serum TRX levels and heart failure and indicate that TRX is induced by acute inflammatory stimuli in the heart. Additional study is required to determine the pathophysiologic role of TRX secreted in heart failure.

Protective Role of Thioredoxin Against Heart Injury

Turoczi et al²⁸ found in ex vivo working rat heart that reperfusion of ischemic myocardium downregulated TRX expression. However, TRX was upregulated in the adapted myocardium after cyclic episodes of ischemia reperfusion. The adaptive protection was abolished by a TRX inhibitor, *cis*-diammine-dichloroplatinum. In addition, TRX-overexpressing mouse hearts had improved postischemic ventricular recovery and reduced myocardial infarct size compared with wild-type hearts. The results implicate a protective role for endogenous TRX in ischemic myocardium. Other authors found important roles for exogenous TRX in decreasing reperfusion-induced arrhythmias²⁹ and in decreasing adriamycin-induced cytotoxic injury.³⁰ These results suggest that both endogenous and exogenous TRX has a protective role against ROS-mediated cardiotoxicity.

Thioredoxin-Binding Proteins

Another key mechanism by which TRX mediates cell protection is via binding to signaling molecules and modulating their function. Below we discuss several examples with important cardiovascular effects.

Apoptosis Signal-Regulating Kinase 1

Apoptosis signal-regulating kinase 1 (ASK1), a mitogen-activated protein kinase kinase kinase, plays essential roles in stress-induced apoptosis.³¹ ASK1 is activated by many stress- and cytokine-related stimuli and activates c-Jun NH₂-terminal kinase (JNK) and p38 mitogen-activated protein kinase. Our group has studied ASK1 because of our interest in the atheroprotective mechanisms of steady laminar flow. Using both in vitro cultured ECs^{32,33} and ex vivo intact vessels,³⁴ we have demonstrated that inhibition of tumor necrosis factor (TNF)-mediated activation of the ASK1-JNK pathway is one possible mechanism by which steady laminar flow is atheroprotective. Through genetic screening for ASK1-binding proteins, Saitoh et al³⁵ found that TRX bound directly to the N-terminus of ASK1 and inhibited ASK1 kinase activity as well as ASK1-dependent apoptosis. The interaction between TRX and ASK1 was regulated by TRX redox status, because the interaction was observed only under reducing conditions and the redox-inactive mutant of TRX (mutated at cysteines 32 and 35) did not bind to ASK1.

Recently, Liu and Min³⁶ demonstrated redox-independent inhibition of ASK1 by TRX in cultured bovine aortic ECs. They first showed that overexpression of wild-type TRX induced ASK1 ubiquitination and degradation. A single mutation of TRX at the catalytic site (Cys32 or Cys35) also retained binding activity for ASK1 and the ability to induce ASK1 ubiquitination/degradation. These results suggest that association of TRX with ASK1 through a single cysteine is necessary and sufficient for TRX to induce ASK1 ubiquitination/degradation, leading to inhibition of ASK1-induced apoptosis.

Vitamin D₃-Upregulated Protein 1

Vitamin D₃-upregulated protein 1 (VDUP1) was originally identified in HL-60 leukemia cells treated with 1,25-dihydroxyvitamin D₃.³⁷ Thereafter, Nishiyama et al³⁸ isolated VDUP1 as a TRX-binding protein using a yeast two-hybrid system. Biochemical analysis showed that VDUP1 inhibits TRX activity by interacting with the catalytic site of TRX, suggesting that VDUP1 is an endogenous inhibitor of TRX.^{38,39} Han et al⁴⁰ suggested that VDUP1 exerts an antitumor effect, because VDUP1 expression was reduced in human tumor tissues and upregulation of VDUP1 by 1,25-dihydroxy vitamin D₃ or transforming growth factor- β inhibited tumor cell growth.

Little is known about the function of VDUP1 in cardiovascular tissues. Wang et al⁴¹ recently demonstrated in rat neonatal cardiomyocytes that exposure to biomechanical strain suppressed VDUP1 expression followed by increases in TRX activity. Overexpression of VDUP1 sensitized cells to H₂O₂-induced apoptosis, whereas overexpression of TRX protected against injury. Schulz et al²⁰ showed in human aortic VSMCs that PDGF and H₂O₂ suppressed VDUP1 expression, with increases in TRX activity and DNA synthesis. Conversely, overexpression of VDUP1 abolished PDGF-induced TRX activity and DNA synthesis. These results suggest that VDUP1 has proapoptotic effects in cardiomyocytes and VSMCs through the suppression of TRX activity. In summary, it seems that regulation of VDUP1 is a critical molecular switch in the transduction of prooxidant mitogenic signals.

Using a perfused vessel culture system, we recently found in endothelium of intact rabbit aorta that exposure to physiological shear stress (12 dyne/cm² for 24 hours) decreased VDUP1 expression and increased TRX activity (H.Y., B.C.B, unpublished data, 2003). Physiological shear stress inhibited TNF stimulation of JNK, p38, and VCAM-1 expression in aortic ECs.³⁴ In cultured human umbilical vein ECs (HUVECs), decreasing VDUP1 by RNA interference increased TRX binding to ASK1 and inhibited TNF stimulation of JNK, p38, and VCAM-1 expression (H.Y., B.C.B, unpublished data, 2003). These data demonstrate a novel mechanism for the atheroprotective effects of physiological shear stress via decreased VDUP1 and suggest that VDUP1 may be a proatherosclerotic mediator.

Posttranslational Modifications of Thioredoxin Function

TRX contains five cysteines. Cysteines 32 and 35 are in the redox-regulatory domain of TRX, which is highly conserved.

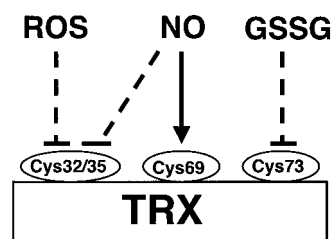


Figure 2. Posttranslational regulation of TRX.

Cysteines 62, 69, and 73 are also structurally important cysteines.^{42,43} Posttranslational modifications of the cysteines by oxidation, S-nitrosylation and glutathionylation, significantly affect TRX function (Figure 2).

Oxidation of the sulfhydryl groups of cysteines 32 and 35 forms a disulfide bond that alters TRX conformation and dissociates TRX from ASK1. S-nitrosylation is the reversible covalent binding of NO to a SH group of a reactive cysteine.⁴⁴ Different cysteines exhibit varying sensitivity for S-nitrosylation. Several studies demonstrated that TRX functions can be regulated by S-nitrosylation.^{45,46} In human embryonic kidney 293 cells, which do not contain eNOS, the redox regulatory domain (Cys32/35) is likely to be sensitive to S-nitrosylation.⁴⁶ For NO-producing cells, such as HUVECs, cysteine 69 has been identified to be S-nitrosylated.⁴⁵ S-nitrosylation of TRX at cysteine 69 increased the redox-regulatory activity of TRX. Interestingly, S-nitrosylation of TRX at cysteine 69 in part accounts for the antiapoptotic capacity of TRX in HUVECs, whereas mutation of cysteine 69 had no protective effect in human embryonic kidney 293 cells, underscoring the role for cysteine 69 in NO-producing cells.

Glutathione and TRX are two major reducing systems that maintain the redox balance of the cell. Previously, the two systems were considered to be parallel redox systems, because these two systems differ greatly in their functions and responses to stress. However, recently Casagrande et al⁴⁷ demonstrated that under conditions of oxidative stress, TRX can react with glutathione at cysteine 73 to form TRX-glutathione-mixed disulfides, termed glutathionylation. Glutathionylation of TRX inhibits its enzymatic activity and function. This study suggests that a crosstalk between the glutathione and the TRX system may act as an indicator of the redox status of the cell.

Other Atheroprotective Antioxidant Systems: Glutathione Peroxidase, Glutathione Reductase, and Glutaredoxin

Glutaredoxin (GRX) and glutathione cooperate with TRX to modulate redox states in a cell-specific manner. Takeshita et al⁴⁸ showed that laminar flow for 24 hours upregulated glutathione peroxidase (GPx) expression and activity in cultured bovine aortic ECs. Ennezat et al⁴⁹ found that physical training restores NO-mediated EC dysfunction in patients with chronic heart failure through increased expression of Cu/Zn SOD and GPx. Because SOD converts superoxide anion to H₂O₂ and because GPx, a ubiquitously expressed antioxidative selenoprotein, reduces H₂O₂ to H₂O, the

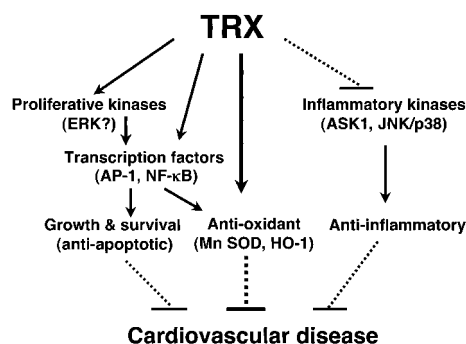


Figure 3. Proposed model for functions of TRX in the cardiovascular system.

ability of flow to induce these antioxidants may be an important mechanism by which flow protects cells against oxidative stress and atherogenesis.

Our group⁵⁰ showed in bovine lung microvascular ECs that steady laminar flow decreased H₂O₂-induced JNK activation. We found that flow significantly increased the ratio of reduced glutathione to oxidized glutathione, consistent with an increase in glutathione reductase (GR) activity. Overexpression of GR mimicked the effect of flow to inhibit JNK activation, suggesting that flow activation of GR, an important regulator of the intracellular redox state of glutathione, also exerts a protective mechanism against oxidative stress and atherogenesis.

GRX, a member of a family of thiol-disulfide oxidoreductases, is a ubiquitously expressed small cytosolic protein that acts as a cytoprotective antioxidant. Like TRX, GRX catalyzes the reduction of protein disulfide bonds by utilizing a Cys-Pro-Tyr-Cys-active site. Okuda et al¹⁵ showed in atherosclerotic coronary arteries that infiltrating macrophages within the neointima highly expressed GRX, which correlated with the generation of ROS. They also found that H₂O₂ stimulated the expression of GRX in cultured human coronary VSMCs. These results suggest the possible involvement of GRX in antioxidant protection in human coronary arteries. Additional work is necessary to determine if shear stress exerts atheroprotective effects via regulation of GRX expression or activity.

Conclusions

In this review, we have focused on the functional regulation of cardiovascular systems by TRX and its associated proteins (Figure 3). Important future questions include the specific roles of TRX-1 and TRX-2, the relative roles of TRX and GRX, and the regulatory mechanisms (including physiological inducers) of VDUP1. Because the modulation of cellular redox balance by ROS is critically important in the pathogenesis of cardiovascular disorders and TRX exerts important protective roles against ROS, it seems likely that TRX is a promising target for clinical therapy.

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