Protective Mechanism of Thioredoxin-1 against Atherosclerotic Endothelial Injury Induced by Ox-LDL

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ABSTRACT

Atherosclerosis is a chronic inflammation disease that is initiated by endothelial cell injury. Hyperlipidemia is an independent risk factor for atherosclerosis. Oxidized low-density lipoprotein (ox-LDL) is a major component of hyperlipidemia and contributes to atherosclerosis. Ox-LDL and ox-LDL-related reactive oxygen species (ROS) and inflammation have deleterious effects on vascular endothelial cells. Previous studies have demonstrated that thioredoxin-1 (Trx) is one of the key regulators of intracellular redox, which may play a role in the pathogenesis of atherosclerosis. This review will summarize the protective mechanisms of Trx on endothelial injury induced by ox-LDL.

Introduction

Atherosclerosis is the main cause of coronary heart disease, cerebral infarction, peripheral vascular diseases. It is a chronic inflammation disease that is initiated by endothelial cell injury. Lipid metabolic disorders are the basis of atherosclerotic lesions. Abundant data link hypercholesterolaemia to atherogenesis. However, recently we have demonstrated that inflammatory mechanisms couple dyslipidaemia to atheroma formation. Over the past decade, we have come to appreciate a prominent role for inflammation in atherosclerosis and its complications, and put forward the notion that atherosclerosis is a chronic inflammation. In early atherogenesis, pro-inflammatory chemokines recruit leukocytes and induce expression of pro-inflammatory cytokines which contribute greatly to atherogenesis1.

Among the factors contributing to atherosclerosis, reactive oxygen species (ROS) is a pivot to regulate many signal pathways. ROS are normal products of cellular redox metabolism. It is believed that ROS serve as subcellular messengers in gene regulatory and signal transduction pathways. Whereas when ROS are generated more than scavenging, they accumulated. Once the cellular redox homeostasis is broken out, oxidative stress occurred. ROS can regulate some inflammatory gene's expression via signal transduction pathways in addition to direct damnification of cells, and lead to a series of pathological and physiological courses. Endothelial dysfunction and activation are crucial factors to initiate atherosclerosis which can be contributed by various factors such as ox-LDL, advanced glycation end-products (AGEs), and Angiotensin-2 etc2. Ox-LDL disrupts the antioxidant and secretory activities of vascular endothelium3, and
stimulates vascular O²⁻ formation presumably via activation of the membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase⁴, thus being considered as an important risk factor for endothelial injury. A large body of evidence suggests that increase of ROS is involved in the genesis of both endothelial vasodilator dysfunction and endothelial activation⁵. Of the several potential sources of ROS in vasculature, the endothelial NADPH oxidase family is a major contributor to ROS in atherosclerosis.

ROS level is tightly controlled in vasculature. Thioredoxin (Trx) is small-molecule protein, which exists widely in eukaryotic and prokaryotic organisms. Trx, thioredoxin reductase (TrxR) and NADPH comprise the thioredoxin system, which is one of the most important thiol oxidoreductase systems. Human thioredoxin is a 12-kDa protein with redox-active dithiol, its active site -Cys-Gly-Pro-Cys- contains two cysteines (dithiol) undergoing reversible redox change between an oxidized disulfides (-S-S-) and a reduced dithiol (-SH, -SH)⁶. Currently, three isoforms of human Trx have been identified. Trx1 is a 104-amino acid protein that is typically found in the nucleus of normal endometrial stromal cells, tumor cells, and primary solid tumors⁷. Trx2 usually exists in mitochondria, it is a 166-amino acid protein that contains a 60-amino acid NH₂-terminal translocation sequence⁸. The third isoform SpTrx is highly expressed in testicular tissue, which only exists in the Golgi apparatus in mammalian spermatocytes and sperm cells⁹. Unless otherwise indicated, Trx refers to Trx1 in this review. Evidence indicates that Trx may play a role in the pathogenesis of atherosclerosis⁴,³⁰,¹¹,¹²,¹³,¹⁴, including the control of cellular redox balance, and the therapeutic approaches targeting the Trx system are emerging.

This review will explore the protective mechanisms of Trx on endothelial injury induced by ox-LDL, and may provide new clues for atherosclerosis prevention and therapy.

**Trx reduces the activity of NADPH oxidase**

ox-LDL is well known to play a crucial role in the initiation and progression of atherosclerosis, which can be considered an inflammatory disease¹⁵. Under the stimulation of ox-LDL, vascular endothelial cells can increase the generation of ROS and the expression of inflammatory proteins¹⁰,¹¹. ROS-induced oxidative stress is closely related to endothelial dysfunction in humans and rats⁵,¹⁶,¹⁷. Of the several potential sources of ROS in the vasculature, the endothelial NADPH oxidase family is a major contributor to ROS associated with atherosclerosis¹⁸. Nox4 and Nox2 are functional NADPH oxidases isoforms, expressed in endothelial cells. They are the major sources of ROS in endothelial cells and superabundant ROS are implicated in both vasodilator dysfunction and the modulation of redox-sensitive signaling pathways¹⁹. P22phox, a membrane associated component of NADPH oxidase, co-localizes with and directly binds to Nox4. The protein level and stability of p22phox have been reported to be necessary for Nox4 activity²⁰. Nox4 does not require the binding of cytosolic proteins (p47phox, p67phox, and Rac1) for ROS production and instead produces ROS constitutively²¹. Thus, p22phox may be an important regulator of Nox4. Nox2 is another isoform of NADPH oxidase, which also exists in endothelial cells. Nox2 is a highly glycosylated protein, and its activation requires interactions with other membrane (p22phox) and cytosolic (p47phox, p67phox, p40phox, and Rac1/2) components. Rac1 is a cytosolic component of NADPH oxidase, which can bind to Nox2 and affect Nox2 activity. A previous study indicated that Trx levels are associated with NADPH oxidase activity in vivo and in vitro¹⁵. Recently, our lab reported that Trx decreased NADPH oxidase activity in ox-LDL-stimulated human umbilical vein endothelial cells (HUVECs) by detecting NADPH consumption in the NADPH (100 mM) incubated homogenate of supernatant yielded the plasma membrane-enriched fractions. Genetic gain-of-function and loss-of-function studies showed that Trx suppressed ox-LDL induced Nox4 and p22phox expression. A co-immunoprecipitation assay indicated that Trx1 decreased Nox4-p22phox complex level during ox-LDL stimulation. Transient transfection of Nox4 and p22phox significantly increased intracellular ROS generation, which could be blocked by Trx overexpression. In addition, Trx overexpression also prevented ox-LDL-induced Nox2 and Rac1 protein levels (Figure 1).

Our data indicated that Trx inhibited NADPH activity
via suppressing Nox4, ph22phox, Nox2, Rac1 expression, and decreasing the level of Nox4-p22phox complex in ox-LDL treated HUVECs and may prevent the initiation of atherosclerosis by attenuating exceeding ROS production12.

**Trx regulates AP-1 and Smad3 pathways**

Trx regulates a number of redox-sensitive cellular signal transduction molecules and transcription factors including nuclear factor-kappa B (NF-kB), activator protein-1 (AP-1), tumor protein 53 (p53), PEBP222, Smad311 and others. Redox Factor 1 (Ref-1) is a 36.5 kDa protein known as apurinic/apyrimidinic endonuclease (APE1) and was first described as an important enzyme in the base excision repair (BER) pathway. Moreover, Ref-1 has a separate N-terminal domain with redox activity23. Its activity has been shown to enhance the DNA binding ability of transcription factors such as NF-κB24,25, p5326, hypoxia inducible factor-1α (HIF-1α)27 and AP-128. We focused on AP-1 pathways. Trx has been shown to bind to Ref-1 in vitro and through mammalian two-hybrid assays in Cos-7 cells29. Other studies have further implicated that Trx can cooperate with Ref-1 within the nucleus of mammalian cells to enhance AP-1 activity22,29. Our lab reported that overexpression of Trx in ox-LDL stimulated endothelial cells inhibited c-Jun translocation into the nucleus, knockdown of Trx extremely enhanced nuclear Ref-1 expression and increased the DNA binding activity of AP-1. Luciferase assay also certified that Trx inhibited AP-1 activity in mammalian cells to enhance AP-1 activity22,30. Our lab reported that overexpression of Trx in ox-LDL stimulated endothelial cells inhibited c-Jun translocation into the nucleus, knockdown of Trx extremely enhanced nuclear Ref-1 expression and increased the DNA binding activity of AP-1. Luciferase assay also certified that Trx inhibited AP-1 activity in ox-LDL treated endothelial cells10. The above experimental results seem to be inconsistent, but this may be due to different cell types and stimulus conditions. The expression of Trx in human vascular endothelial cells affects the nuclear translocation of Ref-1 and AP-1 subunit c-Jun, thereby inhibiting the activity of AP-1.

Smad transcription factors are specific downstream mediators of the transforming growth factor β (TGF-β) signaling pathway. Smad3 belongs to receptor-regulated Smads (R-Smads) and can be activated by TGF-β and activin receptors. In the process of atherosclerotic plaque formation, TGF-β/Smad3 signaling pathway plays a role of anti-inflammatory protection. TGF-β prevents the ox-LDL-induced expression of adhesion molecules31 and contributes to plaque stabilization32. Additionally, in vascular cells, cholesterol suppressed TGF-β signaling by increasing lipid rafts and the caveolea accumulation of TGF-β receptors33. Although the TGF-β/Smad pathway has been shown to have protective, anti-inflammatory effects on cells that are important to atherosclerotic lesion formation [8], it remains unclear how Smad3 contributes to ox-LDL stimulation in HUVECs. Recently, we found that overexpression of functional Trx significantly inhibited VCAM-1 and ICAM-1 expression in ox-LDL stimulated HUVECs, which can be abolished by SIS3, a specific inhibitor of Smad3 phosphorylation. Further detected, we found that Trx enhanced Smad3 phosphorylation and nuclear translocation. A co-immunoprecipitation assay indicated that Smad3 continued to interact with Trx. This interaction might be responsible for the further nuclear translocation of active Smad3 (phosphorylated Smad3, pSmad3) in Trx-overexpressing HUVECs and activation of the Smad3 signaling pathway which presents a crucial role in atherogenesis11 (Figure 2).

**Trx inhibits the expression of inflammatory proteins**

Atherosclerosis is a hypercholesterolemia-based and oxidative stress-associated chronic inflammatory disease. Endothelial cells express vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) in response to cholesterol feeding selectively in areas prone to lesion formation [5, 6], which seem to play a major role in recruiting monocyte to the atheroma. Monocyte chemoattractant protein-1 (MCP-1) is a key mediator of monocyte infiltration and plays a crucial role in the pathogenesis of atherosclerosis. The inflammatory factors link the occurrence of hyperlipidemia and atherosclerosis, which are important initiators of atherosclerosis. Our study indicated that Trx could suppress VCAM-1, ICAM-1 expression and MCP-1 secretion in ox-LDL stimulated HUVECs10,11. Therefore, Trx may play an essential role of atheroprotection (Figure 2).

**Summary**

In conclusion, our series of studies put forward new mechanisms of Trx for protecting endothelial cells against ox-LDL induced atherosclerosis, which may provide new
clues for atherosclerosis prevention and treatment at the level of endothelial cells.

References