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Original Contribution

Thioredoxin-1 suppresses lung injury and apoptosis induced by diesel exhaust particles (DEP) by scavenging reactive oxygen species and by inhibiting DEP-induced downregulation of Akt

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Abstract

Diesel exhaust particles (DEP) are reactive oxygen species (ROS)-inducing toxic agents that damage lungs. Thioredoxin-1 (Trx-1) is a thiol protein with antioxidant and redox-regulating effects. In this study, we demonstrate that Trx-1 scavenges ROS generated by DEP and attenuates the lung injury. Intratracheal instillation of DEP resulted in the generation of more hydroxyl radicals in control mice than in human Trx-1 (*h*Trx-1)-transgenic mice as measured by noninvasive L-band in vivo electron spin resonance. DEP caused acute lung damage with massive infiltration of inflammatory cells in control mice, but much less damage in *h*Trx-1-transgenic mice. The *h*Trx-1 transgene protected the mice against DEP toxicity. To investigate further the molecular mechanism of the protective role of Trx-1 against DEP-induced lung injury, we used *h*Trx-1-transfected L-929 cells and recombinant *h*Trx-1 (*rh*Trx-1)-pretreated A-549 cells. DEP-induced ROS generation was suppressed by *h*Trx-1 transfection or pretreatment with *rh*Trx-1. Endogenous Trx-1 expression was induced by DEP in control cells. The downregulation of Akt phosphorylation by DEP resulted in apoptosis, which was prevented by Trx-1. Moreover, an Akt inhibitor canceled this protective effect of Trx-1. Collectively, the results suggest that Trx-1 exerts antioxidant effects in vivo and in vitro and that this plays a role in protection against DEP-induced lung damage by regulating Akt-mediated antiapoptotic signaling.

Keywords: Thioredoxin-1; Reactive oxygen species; Diesel exhaust particles; Lung injury; Noninvasive L-band electron spin resonance; Free radicals

Thioredoxin-1 (Trx-1) is a multifunctional small protein (12 kDa) with thiol-reducing activity at its conserved active site: Cys-Gly-Pro-Cys [1,2]. Several proteins share this active site sequence, and collectively they make up the Trx family. Trx-1 is a cytosolic protein, whereas Trx-2 is specific to mitochondria [3]. Human Trx-1 was originally cloned as a cytokine-like factor named adult T cell leukemia-derived factor (ADF) produced by human T cell leukemia virus type I-transformed T cells [4]. Its promoter region contains an oxidative-responsive element (ORE), an antioxidant-responsive element (ARE), a xenobiotic-responsive element, a cyclic-AMP-responsive element, and SP-1 [5]. Trx-1 is expressed in response to various forms of stress

Abbreviations: A-549, human lung epithelial cell line; ADF, adult T cell leukemia-derived factor; Akt, protein kinase-B; AP-1, activator protein-1; ARE, antioxidant-responsive element; CAT-1, 4-trimethylammonium-2,2,6,6-tetramethylpiperidine-1-oxyl; DCFH-DA, 2′,7′-dichlorofluorescein diacetate; DEP, diesel exhaust particles; DMEM, Dulbecco's modified Eagle's medium; DM, double mutant; ESR, electron spin resonance; G418, neomycin; *h*, human; L-929, lung fibrosarcoma cell line; NF-κB, nuclear factor κB; ORE, oxidative-responsive element; PBS, phosphate-buffered saline; PI, propidium iodide; PI3-K, phosphatidylinositol 3-kinase; $PM_{2.5}$, particulate matter <2.5 μm; *rh*, recombinant human; ROS, reactive oxygen species; Trx-1, thioredoxin-1; Trx-2, thioredoxin-2; WT, wild type.

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such as viral infection, ischemia–reperfusion, UV light, X-ray irradiation, and H_2O_2 [6]. Inside the cell, Trx-1 is involved in a wide range of cellular responses, including cell proliferation, apoptosis, and cytoprotection by scavenging reactive oxygen species (ROS) and activation of transcriptional factors [7–9]. The reducing activity of NF-κB and AP-1 is 1000 times more efficient than that of another redox regulator, glutathione [10]. Trx-1-knockout mice die in the embryonic stage [11]. Therefore, Trx-1 is essential for redox signaling in cells or for some other essential function.

Overexpression of human Trx-1 in *h*Trx-1-transgenic mice confers resistance against various forms of oxidative stress compared with C57BL/6 control mice. Human Trx-1-transgenic mice are more resistant to influenza virus-induced pneumonia [12] and inflammatory cytokine- or bleomycin-induced acute lung injury [13]. These reports suggest that Trx-1 has beneficial effects on oxidative stress-associated disorders.

Diesel exhaust particles (DEP) derived from diesel engine-powered automobiles and industrial machines are toxic air pollutants and a major source of atmospheric particulate matter $(PM)_{2.5}$ (particles <2.5 μm). $PM_{2.5}$ have an adverse effect on health, because they can reach the alveoli and deposit there [14]. Carbon nuclei of DEP contain various toxic compounds such as aliphatic hydrocarbons, polycyclic aromatic hydrocarbons, nitroaromatic hydrocarbons, heterocyclics, quinones, aldehydes, pyrenes, and some trace heavy metals [15–20]. DEP have recently become a major worldwide health concern and may cause acute and chronic bronchitis, pulmonary fibrosis, and lung cancer [21,22]. As an immediate effect, DEP cause acute lung injury [23–25] with airway neutrophilic inflammation [26,27]. Organic compounds in DEP such as quinones induce the generation of free radicals [28,29]. It has been also proposed that the pathogenesis of DEP-induced pulmonary injury is initiated by free radicals [30].

In this study, by applying the direct in vivo electron spin resonance (ESR) technique, we demonstrate that the overexpression of *h*Trx-1 in transgenic mice attenuates DEP-induced lung damage by scavenging free radicals. We also show that the molecular mechanism by which Trx-1 protects cells against DEP is at least partly explained by Akt-dependent antiapoptotic signaling.

Materials and methods

Reagents

DEP were kindly provided by Dr. Masaru Sagai (Aomori University of Health and Welfare, Aomori, Japan). The particles were dissolved in phosphate-buffered saline (PBS; pH 7.4) containing 0.05% Tween 80 (Sigma, St. Louis, MO, USA) (hereafter referred to as vehicle) by sonicating three times (1 min each time) using a water-bathed ultrasonic sonicator (Iuchi, Osaka, Japan). The anesthetic agent, urethane, was purchased from Sigma and dissolved in normal saline (Otsuka Pharmaceuticals, Tokushima, Japan). The 4-trimethylammonium-2,2,6,6-tetramethylpiperidine-1-oxyl (CAT-1) probe and 2′,7′-dichlorofluorescein diacetate (DCFH-DA) were purchased from Molecular Probes (Eugene, OR, USA). The Akt inhibitor 1 L-6-hydroxymethylchiroinositol 2-(*R*)-2-*O*-methyl-3-*O*-octadecyl carbonate (which inhibits PI3-K) and propidium iodide (PI) were purchased from Calbiochem (Tokyo, Japan). Anti-phospho-Akt (Ser-473) and anti-Akt rabbit polyclonal antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Recombinant human Trx-1 (*rh*Trx-1) was prepared as previously described and provided by Ajinomoto, Inc. (Kawasaki, Japan) [31]. Six times His-tagged wild-type recombinant human Trx-1 (WT-*rh*Trx-1) and double-mutant (DM)-*rh*Trx-1 were prepared as previously reported [32]. In DM-*rh*Trx-1, the cysteines, which are located at positions 32 and 35 in the active sites, were replaced with serines.

Animals

Age- and sex-matched C57BL/6 mice were purchased from Clea (Tokyo, Japan). Human Trx-1-transgenic mice were generated from the C57BL/6 mice using a transgene composed of the β-actin promoter and *h*Trx-1 gene as described previously [33]. All the animals were 8 to 8.5 weeks of age. The mice were humanely treated in a temperature- (25°C) and humidity-controlled room and fed commercial feed (Oriental Yeast, Tokyo, Japan). The animal research committee of the Institute for Virus Research, Kyoto University (Kyoto, Japan), approved all of the animal experiments reported in this paper.

Cell culture

A human Trx-1-transfected clone of L-929 cells (L929-ADF; murine fibrosarcoma cells) and a control clone (L-929-Neo1) were produced as described previously [34]. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Nissui, Tokyo, Japan) with the selection marker G418 (200 μg/ml; Nacalai Tesque, Kyoto, Japan). A-549 human lung epithelial cells were cultured in Ham's F-12 medium (Nacalai Tesque). Both DMEM and Ham's F-12 medium contained 10% heat-inactivated fetal calf serum and antibiotics (penicillin 100 U/ml and streptomycin 100 μg/ml). Cells were incubated at 37°C with 5% CO_2 in air.

Noninvasive L-band in vivo ESR

The control and *h*Trx-1-transgenic mice were instilled with 0.05 mg/mouse DEP dissolved in 50 μl vehicle or an equal volume of vehicle through the trachea under anesthesia with an intramuscular injection of 20% urethane solution (7 μl/g body wt). Twenty-four hours after the treatment with DEP or vehicle, the mice were administered