ANTIOXIDANTS & REDOX SIGNALING
Volume 6, Number 1, 2004

Forum Rapid Letter

Intravenous Administration of Thioredoxin Decreases Brain Damage Following Transient Focal Cerebral Ischemia in Mice

ITARO HATTORI,[1,2] YASUSHI TAKAGI,[2] HAJIME NAKAMURA,[1] KAZUHIKO NOZAKI,[2] JIE BAI,[1] NORIHIKO KONDO,[1] TOSHIYUKI SUGINO,[2] MASAKI NISHIMURA,[2] NOBUO HASHIMOTO,[2] and JUNJI YODOI[1]

ABSTRACT

Thioredoxin (TRX) is induced by a variety of oxidative stimuli and shows cytoprotective roles against oxidative stress. To clarify the possibility of clinical application, we examined the effects of intravenously administered TRX in a model of transient focal cerebral ischemia in this study.Mature male C57BL/6j mice received either continuous intravenous infusion of recombinant human TRX (rhTRX) over a range of 1–10 mg/kg, bovine serum albumin, or vehicle alone for 2 h after 90-min transient middle cerebral artery occlusion (MCAO). Twenty-four hours after the transient MCAO, the animals were evaluated neurologically and the infarct volumes were assessed. Infarct volume, neurological deficit, and protein carbonyl contents, a marker of protein oxidation, in the brain were significantly ameliorated in rhTRX-treated mice at the dose of 3 and 10 mg/kg versus these parameters in control animals. Moreover, activation of p38 mitogen-activated protein kinase, whose pathway is involved in ischemic neuronal death, was suppressed in the rhTRX-treated mice. Further, rhTRX was detected in the ischemic hemisphere by western blot analysis, suggesting that rhTRX was able to permeate the blood–brain barrier in the ischemic hemisphere. These data indicate that exogenous TRX exerts distinct cytoprotective effects on cerebral ischemia/reperfusion injury in mice by means of its redox-regulating activity. *Antioxid. Redox Signal.* 6, 81–87.

INTRODUCTION

OXIDATIVE STRESS is one of the crucial factors in the development of ischemic brain injury (6). During ischemia or ischemia/reperfusion in the brain, excessive reactive oxygen species (ROS) alter or disrupt the balance of the redox potential in cells and lead to protein oxidation, lipid peroxidation, and DNA damage (4, 8).

As therapeutic agents for stroke, various antioxidant compounds have received much attention. Indeed, many studies in experimental animals have documented the ability of antioxidants to reduce ischemic brain injury (21, 38, 41). However, to our knowledge, few antioxidant drugs have been shown to be beneficial in clinical stroke trials (18). Thioredoxin (TRX), a small protein that catalyzes a dithiol/disulfide interchange (14), is induced by various types of oxidative stress, such as x-ray irradiation, ultraviolet light, and hydrogen peroxide (26).TRX has physiologically cytoprotective effects against oxidative stress by scavenging ROS by itself (19) and partly with peroxiredoxin (3). In addition, it plays important roles in cellular signaling via thiol redox control, for example, in the regulation of transcription factors such as nuclear factor-κB and activator protein-1 (13). Further, TRX is secreted from the cells through a unique leaderless pathway (30) and exerts a cytokine-like effect on target cells. Recently, we showed a crucial role for TRX in the nervous system following the application of various stressful stimuli. For example, both protein and mRNA levels of TRX were decreased in the ischemic core and increased in the penumbra during permanent middle cerebral artery occlusion (MCAO) in rats (34).

[1]Department of Biological Responses, Institute for Virus Research, Kyoto University, Kyoto, Japan.
[2]Department of Neurosurgery, Kyoto University Graduate School of Medicine, Kyoto, Japan.

Exogenously administered recombinant TRX was shown to have a neuroprotective effect on murine primary cultured neurons (15). Moreover, both ischemic brain injury and excitotoxic hippocampal injury were attenuated in transgenic mice overexpressing human TRX (hTRX) (35, 37). In this study, we examined the neuroprotective effects of recombinant hTRX (rhTRX) in a murine model of transient focal ischemia, in which rhTRX was delivered intravenously beginning at the start of reperfusion.

MATERIALS AND METHODS

Cerebral ischemia model

Male C57BL/6j mice (Shimizu Laboratory Supplies, Kyoto, Japan) weighing 25–30 g were anesthetized with 1% halothane in 30% O_2/70% N_2O. The right femoral vein was cannulated with a polyethylene tube (PE-10).

Rectal temperature was maintained at 36.5°C by using a homeothermic blanket (Animal Blanket Controller ATB-1100; Nihon Koden, Tokyo, Japan). Focal cerebral ischemia for 90 min was induced by MCAO by using the intraluminal filament technique described previously (22). Regional cerebral blood flow (rCBF) was monitored by laser Doppler flowmetry (Laserflotm; TSI Incorporated). As previously described (16), fiber- optic tips (TSI Incorporated) were affixed to the skull, 3 mm lateral and 2 mm posterior to bregma (designated "ischemic core") and 6 mm lateral and 2 mm posterior to bregma (designated "penumbra") on the ipsilateral hemisphere.

Treatment with rhTRX

rhTRX was produced as previously described (25). Treated animals (n = 8 for each dosage group) received rhTRX at a total dose of 1, 3, or 10 mg/kg by continuous infusion by using a microprocessor-controlled syringe pump (KD Scientific). We chose these concentrations of rhTRX based on the results of previous studies on lung ischemia/reperfusion models (20, 28).

The drug was sterile-filtered, dissolved in 0.4 ml of 0.9% saline, and infused into the right femoral vein for 2 h, beginning just after the start of middle cerebral artery reperfusion. Control animals received an intravenous infusion of bovine serum albumin (BSA, 3 mg/kg) in 0.4 ml of 0.9% saline, saline alone, or no infusion (each n = 8). During infusions, the animals were awakened from anesthesia and allowed full freedom of movement. In some experimental animals, the left femoral artery was cannulated for blood pressure measurement by using an RMP-6004M transducer amplifier (Nihon Kohden) and for blood gas determination by using a blood gas–pH analyzer (Corning 248; Ciba–Corning Diagnostics, Tokyo, Japan). At the end of infusions, animals were reanesthetized to remove the cannulae and were returned to their cages.

Neurological assessment

Twenty-four hours after the transient MCAO, the neurological deficits in the mice were assessed by a masked observer. We evaluated the severity of the neurological deficits by using a four-tiered grading system previously described (11): 0, no observable neurological deficits (normal); 1, failure to extend forepaw (mild); 2, circling to the left side (moderate); and 3, loss of walking or righting reflex (severe).

Measurement of infarct size

Twenty-four hours after the transient MCAO, the mice were deeply anesthetized with sodium pentobarbital (100 mg/kg i.p.). Their brains were immediately removed and cut into 2-mm coronal sections. These sections were stained with 2% 2,3,5-triphenyltetrazolium chloride (TTC) solution and then fixed in 10% paraformaldehyde. Each infarct area was measured by use of NIH Image analyzer software (version 1.61) to calculate the infarction volume.

Detection of rhTRX

To determine if systemically administered rhTRX could permeate the ischemic brain tissue, we examined brain tissue for the presence of rhTRX by western blotting. Immediately after the 2-h drug infusion, animals were deeply anesthetized and transcardially perfused with phosphate-buffered saline, and then their brains were removed. The two hemispheres of each brain were homogenized, each in 1 ml of ice-cold solubilizing solution [0.5% NP-40, 1 mmol/L Tris-HCl (pH 7.2), 15 mmol/L NaCl, 0.1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 2 μg/ml aprotinin]. Equal high amounts of protein (200 μg/lane), estimated by the Bradford method, were electrophoresed on 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gels and transferred to polyvinylidene difluoride membranes (Immobilon; Millipore). After having been blocked with 5% skim milk, the membranes were incubated with anti-hTRX murine monoclonal antibody, which was earlier proved not to recognize endogenous murine TRX at all (15), and then with peroxidase-linked second antibody (Amersham Pharmacia Biotech). Chemiluminescence was detected with an ECL plus western blot detection kit (Amersham Pharmacia Biotech).

Detection of oxidized proteins

Oxidized protein was detected by using an oxidized protein detection kit (OxyBlot, Intergen). The OxyBlot provides reagents for sensitive immunodetection of carbonyl groups, which is a marker of cellular protein oxidation. With this kit, the carbonyl groups in the protein side chains are derivatized to 2,4-dinitrophenylhydrazone (DNP-hydrazone) by reaction with 2,4-dinitrophenylhydrazine (29, 35). The DNP-derivatized protein samples are then separated on an SDS–PAGE gel of gradient density between 5 and 20% (Real Gel Plate, Biocraft, Tokyo, Japan) and subsequently subjected to western blotting. The amount of carbonyl groups extracted from 18 μg of sample proteins was measured by using the NIH Image analyzer software by comparison with the band density of 2.5 μl of a control DNP-lated protein mixture (OxyBlot, Intergen).

Evaluation of activation of p38 mitogen-activated protein kinase (MAPK)

Western blots were used to measure p38 MAPK activation. Antibody specific for active forms of p38 MAPK (Promega) used in this study was previously described (36). The brains of ischemic hemisphere were homogenized in

0.5 ml of ice-cold buffer containing 2 mmol/L Tris-HCl (pH 7.5), 0.1 mmol/L EDTA, 0.5 mmol/L $MgCl_2$, 0.1 mmol/L dithiothreitol, 20 mg/ml aprotinin, 0.1 mmol/L PMSF, and 0.2 mmol/L sodium orthovanadate. After SDS–PAGE, blotting to membranes, and blocking with 5% skim milk, the membranes were incubated with the primary antibody, and then with secondary antibody (Promega).

Statistical analysis

rCBF, infarct volumes, physiological parameters, and protein carbonyl contents were statistically analyzed by ANOVA with Bonferroni's posthoc analysis (software StatView 5.0). Neurological scores were compared by using the Mann–Whitney *U* test. Values are expressed as the means ± SD, with $p < 0.05$ considered statistically significant.

RESULTS

TRX attenuates brain damage

In the three control groups, which received BSA, saline, and no infusion, infarct volumes were 80.6 ± 14.6, 82.7 ± 14.9, and 79.8 ± 13.9 mm^3, respectively. There was no statistical difference in infarct volumes among these control groups. rhTRX at doses of 3 and 10 mg/kg significantly reduced the infarct volume to 57.9 ± 11.3 and 59.4 ± 8.6 mm^3, respectively, as compared with any of these controls (Fig. 1C). rhTRX at a dose of 1 mg/kg, however, caused no statistically significant reduction in infarct volume. The smaller infarct size in the rhTRX-treated animals was due to sparing of the cortex at the periphery of the penumbra (Fig. 1A).

Neurological scores in noninfused control mice at 24 h after ischemia were 2.25 ± 0.89. These scores were significantly reduced to 1.13 ± 0.84 and 1.14 ± 1.07 for mice receiving rhTRX at doses of 3 and 10 mg/kg, respectively (Fig. 2, each $p < 0.04$, each $n = 8$). However, neurological scores were not significantly different between mice in the 1 mg/kg rhTRX experimental group and those in the noninfused control group. All physiological parameters were similar between treatment groups before MCAO and after administering of treatment (data not shown). rCBFs were not statistically different between rhTRX-treated mice and noninfused control mice at each time point (Fig. 3).

hTRX is detected in the ischemic cerebral hemisphere of rhTRX-treated mice

In the ischemic cerebral hemisphere of rhTRX-treated mice, a hTRX signal was detected as a single band of 13 kDa (Fig. 4). In contrast, no signal for hTRX was detected either in the hemisphere of rhTRX-treated mice contralateral to the MCAO or in the control brain. These data indicate that rhTRX does actually pass into the affected brain tissue from the systemic circulation through the damaged blood–brain barrier (BBB).

rhTRX suppresses the activation of p38 MAPK

During ischemia/reperfusion, p38 MAPK is rapidly induced (36) and is involved in ischemic neuronal death. As judged from the results of western blotting, the level of phosphorylated p38 MAPK was enhanced 2 hours after the start of reperfusion in nontreated mice, but was suppressed if the mice were treated with rhTRX (Fig. 5).

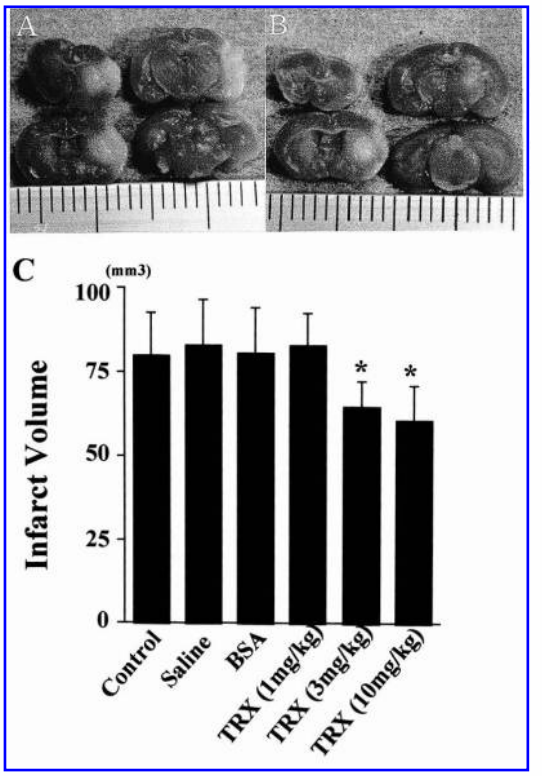

FIG. 1. Infarct volumes 24 h after mice had been subjected to transient MCAO, as assessed by TTC staining. Representative TTC-stained brain sections from a control (nontreated) mouse **(A)** and a rhTRX (3 mg/kg)-treated one **(B)**, as well as infarct volumes in brains of postischemic mice treated with rhTRX, BSA, and saline, and nontreated mice **(C)**, are shown. Bars indicate SD. $n = 8$ for each group; $^*p < 0.05$.

rhTRX suppresses protein oxidation

Oxidative inactivation of enzymes and oxidative modification of proteins by metal-catalyzed oxidation reactions are accompanied by the generation of protein carbonyl derivatives. The protein carbonyl contents at the end of systemic infusion were significantly lower in animals receiving rhTRX at a dose of 3 or 10 mg/kg than in noninfused control animals (Fig. 6A). Between other treatment groups, the protein carbonyl contents were not significantly different at the end of the continuous intravenous infusion (data not shown). The protein carbonyl contents at 24 h after transient MCAO were 201.7 ± 44.1 in noninfused mice and 151.7 ± 29.8 and 148.3 ± 33.7 in rhTRX-treated mice at doses of 3 and 10 mg/kg, respectively (Fig. 6B, % of control, each $p < 0.05$, each $n = 6$).

DISCUSSION

In this study, we showed that intravenously administered rhTRX significantly reduced both infarct volume and neurological deficits in a murine model of transient MCAO. TRX

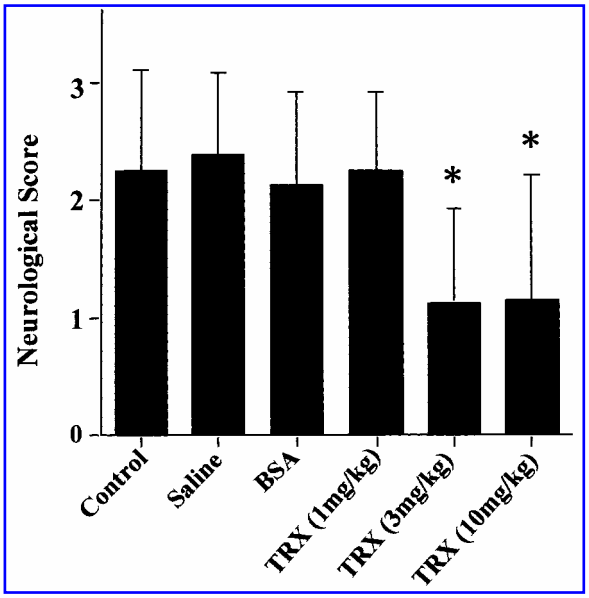

FIG. 2. Neurological deficit score 24 h after transient MCAO. Neurological score was defined in the text from none (0) to most severe (3). Neurological scores were significantly reduced in mice receiving rhTRX at a dose of 3 or 10 mg/kg. These scores were similar between mice in the other experimental group (1 mg/kg rhTRX) and noninfused control mice. Bars show SD. $n = 8$, $*p < 0.04$.

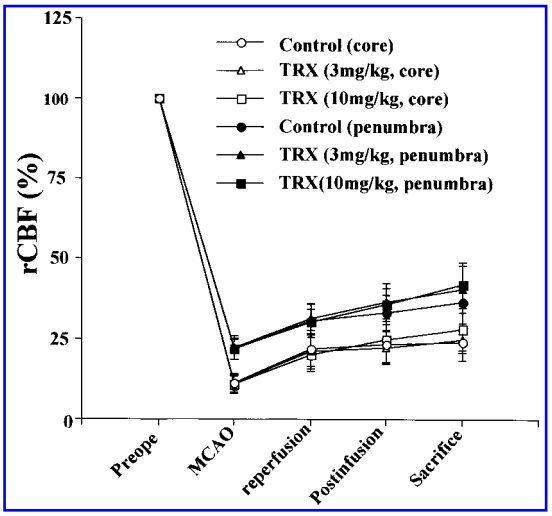

FIG. 3. rCBF in mice subjected to transient MCAO. rCBF was measured over the penumbra and ischemic core of the right middle cerebral artery region in rhTRX-treated and control mice. Preope indicates before MCAO; MCAO, ~3 min after insertion of the occlusive thread into the internal carotid artery; Reperfusion, ~3 min after the thread has been removed; Postinfusion, 2 h after reperfusion; and Sacrifice, 24 h after transient MCAO. The preischemic rCBF was assigned a value of 100%. Subsequent values are presented as a percentage of the preischemic rCBF. Bars show SD. $n = 4$ for each group. No statistical differences were detected between any two groups.

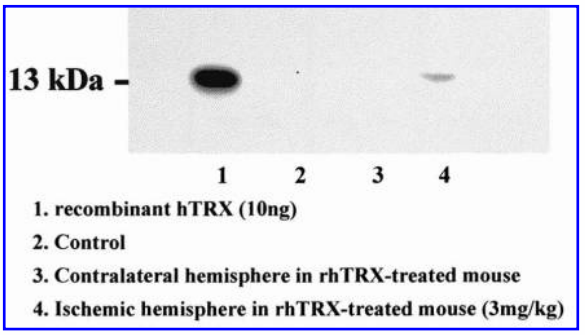

FIG. 4. hTRX detection in the ischemic brain hemisphere of rhTRX-treated mice. The hTRX monoclonal antibody recognizes hTRX as a single band of 13 kDa. In hTRX-treated mice (3 mg/kg), western blot analysis shows a positive band in the cerebral hemisphere of rhTRX-treated mice ipsilateral to the transient MCAO, but not in the contralateral hemisphere. No signal for hTRX is detectable in the sample from the control mice.

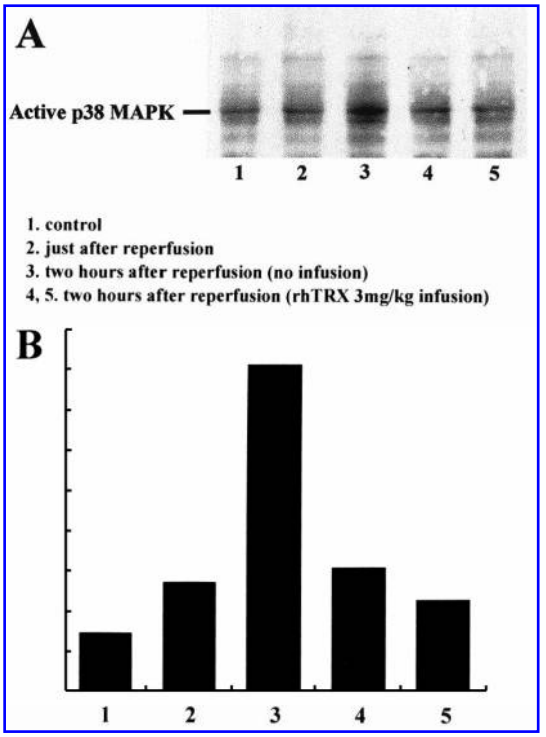

FIG. 5. rhTRX suppresses the activation of p38 MAPK. (A), Western blots of homogenates of nontreated mice before MCAO (lane 1), just after the start of reperfusion (lane 2), and 2 h after the start of reperfusion (lane 3), and of homogenates of rhTRX-treated mice (3 mg/kg) 2 h after reperfusion had begun (lanes 4 and 5). **(B),** Quantitative assessment by densitometric analysis of these western blots. Active p38 MAPK is enhanced just after the start of reperfusion and is increasingly enhanced by 2 h in the control mice; however, active p38 MAPK is suppressed in rhTRX-treated mice in comparison with the level for the control mice.

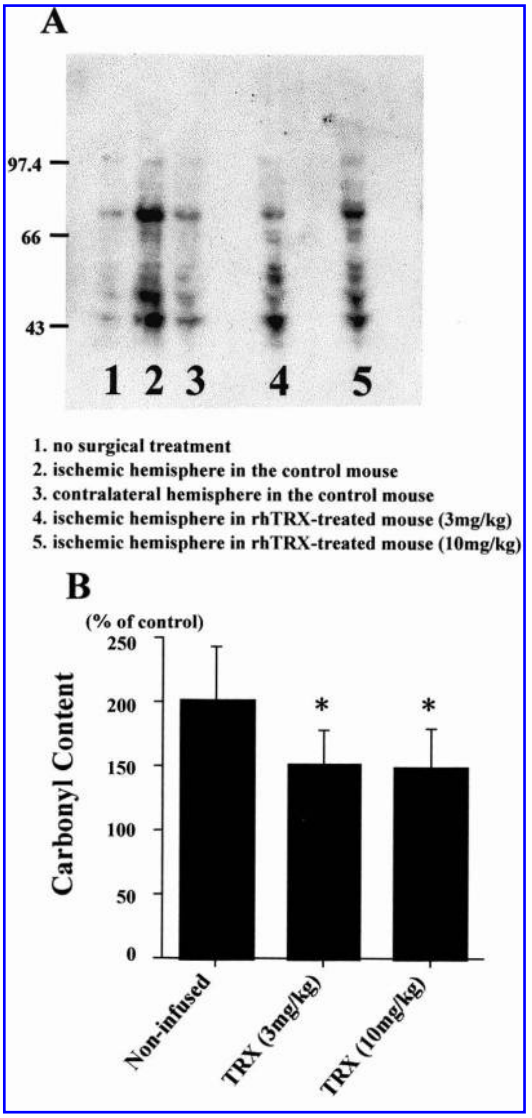

FIG. 6. Changes in protein carbonyl contents after transient MCAO. (A), Two hours after infusion, a lesser amount of DNP moieties was detected in rhTRX-treated mice at a total dose of 3 or 10 mg/kg than in the control mice. **(B),** The protein carbonyl contents at 24 h after MCAO were significantly lower in rhTRX-treated animals at doses of 3 and 10 mg/kg than in noninfused animals (% of control, mean ± SD, $n = 6$ each, $^*p < 0.05$).

exists intrinsically and is induced in the ischemic cerebral hemisphere (35). As extrinsic TRX has apparent beneficial effect on neurons compared with mice treated with vehicle, the evaluation of intrinsic TRX might not be necessary. A conceivable mechanism for preventing ischemic/reperfusion damage by rhTRX administration may include the following: scavenging ROS from brain tissue, cerebral vascular endothelial cells, and neutrophils; and negative regulation of some signal cascade leading to cell death.

During ischemia or ischemia/reperfusion in the brain, excessive ROS are produced by enzymes such as xanthine oxidase in vascular endothelial cells, recruited neutrophils, or an excess of excitatory amino acids (2, 5, 39). Antioxidative stress is one of the key therapeutic strategies for neuronal diseases.

hTRX has the ability to directly scavenge hydrogen peroxide (25), singlet oxygen (19), and hydroxyl radicals (19). Recombinant TRX displays protective activity against a number of toxins and insults in *in vitro* studies, in which excessive ROS are involved in the cytotoxic mechanism (23, 26, 32). In animal models, rhTRX also showed its cytoprotective effects against ischemia/reperfusion-induced lung injury (10, 32). In this study, the protein carbonyl content in the brain of hTRX-treated animals was significantly suppressed in comparison with that of the control animals after transient ischemia, which suggests that exogenous hTRX attenuates oxidative stress by exerting redox-regulating activity including the quenching of ROS.

We also obtained evidence that the administered rhTRX permeated the ischemic cerebral hemisphere through the BBB. During the early reperfusion period after transient focal brain ischemia, endothelial cells exhibit an increased permeability to serum proteins and other high-molecular-weight compounds of molecular weight larger than that of TRX, such as basic fibroblast growth factor and hybridized Cu/Zn superoxide dismutase (SOD) (7, 9, 20). rhTRX that has passed through the BBB may exert direct protective effects on the brain tissue by scavenging ROS. Moreover, we have preliminary data showing that exogenous TRX is taken up by cells (Kondo *et al.*, unpublished observations). Mutant TRX in which two cysteines in the active site are replaced with serines hardly enters into cells, suggesting that the entry of TRX into the cell is dependent on its redox-active site, although the details of the mechanism are still under investigation.

rhTRX in the systemic circulation may be taken up by the brain tissue at least partly and may protect the endothelial cells and brain parenchyma against ischemia/reperfusion-induced oxidative stress by directly scavenging hydrogen peroxide and/or hydroxyl radicals.

In addition to the release of ROS from recruited neutrophils (42), neutrophil adherence to the brain vessel endothelium can cause ischemic brain injury by direct mechanical obstruction of the local blood flow (1). Indeed, some anti-leukocyte adhesion treatments reduce brain injury in animal models of transient focal ischemia (33, 43). Thus, TRX may scavenge ROS released from neutrophils, as well as increase the local cerebral blood flow in the ischemic tissue and decrease infarction by preventing the adhesion of leukocytes to the vascular endothelial membrane. In support of this speculation, we showed earlier by an adhesion assay that rhTRX suppressed the adhesion of neutrophils to human umbilical vein endothelial cells (27) and that systemic administration of rhTRX suppressed extravasation of leukocytes into the inflammatory site (27). Regarding these possibilities, further investigation is required.

TRX acts not only as an antioxidant enzyme, but also as a regulator or modulator in various steps of cellular signaling against oxidative stress. For example, TRX negatively regulates activation of p38 MAPK induced by tumor necrosis factor-α (12). It was also reported that TRX binds to apoptosis signal-regulating kinase-1, a MAPK kinase kinase, and inhibits the apoptosis process (31). We showed that activation of p38 MAPK was suppressed in the rhTRX-treated mice in

this study. Although the mechanism of the antiapoptotic action of extracellular TRX is not yet fully understood, rhTRX may rescue the affected brain via its redox regulation of signal transduction leading to apoptosis resulting from transient focal ischemia.

As for antioxidant therapy, targeting superoxide with SOD has been widely tried (9, 17, 21, 24, 40). However, because of the short half-life of recombinant SOD and the antigenicity of hybrid SOD, which has a long half-life (9), the clinical implication of recombinant SOD is practically limited. In contrast, the half-life of rhTRX in plasma is ~1 h (27), and there is little possibility that human TRX would be antigenic. Therefore, rhTRX is much more likely to succeed as a therapeutic approach to diminish oxidative stress-induced damage in the ischemic brain.

In summary, we demonstrated that intravenous infusion of rhTRX, commencing at the start of reperfusion, reduced infarct volume and improved neurological findings in a murine model of transient focal ischemia. The redox-regulating activity of TRX may contribute to this effect by diminishing oxidative stress. Although further analysis concerning such parameters as therapeutic time window and pharmacodynamics in plasma will help to elucidate the actual mechanism of action of exogenous TRX, the current findings suggest that intravenous infusion of rhTRX represents a potential new approach to the treatment of ischemic stroke.

ACKNOWLEDGMENTS

We thank Y. Kanekiyo for secretarial help. This work was supported by a grant from the program Grants-in-Aid for Research for the Future of the Japan Society for the Promotion of Science and by a grant from Grants-in-Aid for Scientific Research and Special Project Research—Brain Attack of the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

ABBREVIATIONS

BBB, blood–brain barrier; BSA, bovine serum albumin; DNP, 2,4-dinitrophenyl; hTRX, human thioredoxin; MAPK, mitogen-activated protein kinase; MCAO, middle cerebral artery occlusion; PMSF, phenylmethylsulfonyl fluoride; rCBF, regional cerebral blood flow; rhTRX, recombinant human thioredoxin; ROS, reactive oxygen species; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SOD, superoxide dismutase; TRX, thioredoxin; TTC, 2,3,5-triphenyltetrazolium chloride.

REFERENCES

1. Aronow J, Strong R, and Grotta JC. Reperfusion injury: demonstration of brain damage produced by reperfusion after transient focal ischemia in rats. *J Cereb Blood Flow Metab* 17: 1048–1056, 1997.
2. Betz AL. Oxygen radicals in focal cerebral ischemia. *Brain Pathol* 4: 59–65, 1985.
3. Chae HZ, Robinson K, Poole LB, Church G, Storz G, and Rhee SG. Cloning and sequencing of thiol-specific antioxidant from mammalian brain: alkyl hydroperoxide reductase and thiol-specific antioxidant define a large family of antioxidant enzymes. *Proc Natl Acad Sci U S A* 91: 7017–7021, 1994.
4. Chan PH. Role of oxidants in ischemic brain damage. *Stroke* 27: 1124–1129, 1996.
5. Choi DW, Koh JY, and Peters S. Pharmacology of glutamate neurotoxicity in cortical cell culture: attenuation by NMDA antagonists. *J Neurosci* 8: 185–196, 1988.
6. Dinagl U, Iadecola C, and Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci* 22: 391–397, 1999.
7. Fisher M, Meadows ME, Do T, Weise J, Trubetskoy V, Charette M, and Finklestein SP. Delayed treatment with intravenous basic fibroblast growth factor reduces infarct size following permanent focal cerebral ischemia in rats. *J Cereb Blood Flow Metab* 15: 953–959, 1995.
8. Flamm ES, Demopoulos HB, Seligman ML, Poser RG, and Ransohoff J. Free radicals in cerebral ischemia. *Stroke* 9: 445–447, 1978.
9. Francis JW, Ren JM, Warren L, Brown RH Jr, and Finklestein SP. Postischemic infusion of Cu/Zn superoxide dismutase or SOD:Tet451 reduces cerebral infarction following focal ischemia/reperfusion in rats. *Exp Neurol* 146: 435–443, 1997.
10. Fukuse T, Hirata T, Yokomise H, Hasegawa S, Inui K, Mitsui A, Hirakawa T, Hitomi S, Yodoi J, and Wada H. Attenuation of ischemia reperfusion injury by human thioredoxin. *Thorax* 50: 387–391, 1995.
11. Hara H, Friedlander RM, Gagliardini V, Ayata C, Fink K, Huang Z, Shimizu-Sasamata M, Yuan J, and Moskowitz MA. Inhibition of interleukin 1beta converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. *Proc Natl Acad Sci U S A* 94: 2007–2012, 1997.
12. Hashimoto S, Matsumoto K, Gon Y, Furuichi S, Maruoka S, Takeshita I, Hirota K, Yodoi J, and Horie T. Thioredoxin negatively regulates p38 MAP kinase activation and IL-6 production by tumor necrosis factor-alpha. *Biochem Biophys Res Commun* 258: 443–447, 1999.
13. Hirota K, Matsui M, Iwata S, Nishiyama A, Mori K, and Yodoi J. AP-1 transcriptional activity is regulated by a direct association between thioredoxin and Ref-1. *Proc Natl Acad Sci U S A* 94: 3633–3638, 1997.
14. Holmgren A. Thioredoxin. *Annu Rev Biochem* 54: 237–271, 1985.
15. Hori K, Katayama M, Sato N, Ishii K, Waga S, and Yodoi J. Neuroprotection by glial cells through adult T cell leukemia-derived factor/human thioredoxin (ADF/TRX). *Brain Res* 652: 304–310, 1994.
16. Huang Z, Huang PL, Panahian N, Dalkara T, Fishman MC, and Moskowitz M. Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase. *Science* 25: 1883–1885, 1994.
17. Imaizumi S, Woolworth V, Fishman R, and Chan P. Liposome-entrapped superoxide dismutase reduces cerebral infarction in cerebral ischemia in rats. *Stroke* 21: 1312–1317, 1990.

18. Keyser JD, Sulter G, and Luiten PG. Clinical trials with neuroprotective drugs in acute ischaemic stroke: are we doing the right thing? *Trends Neurosci* 22: 535–540, 1999.
19. Kumuda CD and Chanden KD. Thioredoxin, a singlet oxygen quencher and hydroxyl radical scavenger: redox independent functions. *Biochem Biophy Res Commun* 277: 443–447, 2000.
20. Kuroiwa T, Ting P, Martinez H, and Klatzo I. The biphasic opening of the blood–brain barrier to proteins following temporary middle cerebral artery occlusion. *Acta Neuropathol* 68: 122–129, 1985.
21. Liu TH, Beckman JS, Freeman BA, Hogan EL, and Hsu CY. Polyethylene glycol-conjugated superoxide dismutase and catalase reduce ischemic brain injury. *Am J Physiol* 256: H589–H593, 1989.
22. Longa EZ, Weinstein PR, Carlson S, and Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke* 20: 84–91, 1989.
23. Matsuda M, Masutani H, Nakamura H, Miyajima S, Yamauchi A, Yonehara S, Uchida A, Irimajiri K, Horiuchi A, and Yodoi J. Protective activity of adult T cell leukemia-derived factor (ADF) against tumor necrosis factor-dependent cytotoxicity on U937 cells. *J Immunol* 147: 3837–3841, 1991.
24. Matsumiya N, Koehler RC, Kirsch JR, and Traystman RJ. Conjugated superoxide dismutase reduces extent of caudate injury after transient focal ischemia in cats. *Stroke* 22: 1193–1200, 1991.
25. Mitsui A, Hirakawa T, and Yodoi J. Reactive oxygen-reducing and protein-refolding activities of adult T cell leukemia-derived factor/human thioredoxin. *Biochem Biophys Res Commun* 186: 1220–1226, 1992.
26. Nakamura H, Matsuda M, Furuke K, Kitaoka Y, Iwata S, Toda K, Inamoto T, Yamaoka Y, Ozawa K, and Yodoi J. Adult T cell leukemia-derived factor/human thioredoxin protects endothelial F-2 cell injury caused by activated neutrophils or hydrogen peroxide. *Immunol Lett* 42: 75–80, 1994.
27. Nakamura H, Herzenberg LA, Bai J, Araya S, Kondo N, Nishinaka Y, Herzenberg LA, and Yodoi J. Circulating thioredoxin suppresses lipopolysaccharide-induced neutrophil chemotaxis. *Proc Natl Acad Sci U S A* 98: 15143–15148, 2001.
28. Okubo K, Kosaka S, Isowa N, Hirata T, Hitomi S, Yodoi J, Nakano M, and Wada H. Amelioration of ischemia–reperfusion injury by human thioredoxin in rabbit lung. *J Thorac Cardiovasc Surg* 113: 1–9, 1997.
29. Oliver CN, Starke-Reed PE, Stadtman ER, Liu GJ, Carney JM, and Floyd RA. Oxidative damage to brain proteins, loss of glutamine synthetase activity, and production of free radicals during ischemia/reperfusion-induced injury to gerbil brain. *Proc Natl Acad Sci U S A* 87: 5144–5147, 1990.
30. Rubartelli A, Bajetto A, Allavena G, Wollman E, and Sitia R. Secretion of thioredoxin by normal and neoplastic cells through a leaderless secretory pathway. *J Biol Chem* 267: 24161–24164, 1992.
31. Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, and Ichijo H. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK)-1. *EMBO J* 17: 2596–2606, 1998.
32. Sasada T, Iwata S, Sato N, Kitaoka Y, Hirota K, Nakamura K, Nishiyama A, Taniguchi Y, Takabayashi A, and Yodoi J. Redox control of resistance to *cis*-diamminedichloroplatinum (II) (CDDP): protective effect of human thioredoxin against CDDP-induced cytotoxicity. *J Clin Invest* 197: 2268–2276, 1996.
33. Soriano SG, Coxon A, Wang YF, Frosch MP, Lipton SA, Hickey PR, and Mayadas TN. Mice deficient in Mac-1 (CD11b/CD18) are less susceptible to cerebral ischemia/reperfusion injury. *Stroke* 30: 134–139, 1999.
34. Takagi Y, Tokime T, Nozaki K, Gon Y, Kikuchi H, and Yodoi J. Redox control of neuronal damage during brain ischemia after middle cerebral artery occlusion in the rat: immunohistochemical and hybridization studies of thioredoxin. *J Cereb Blood Flow Metab* 18: 206–214, 1998.
35. Takagi Y, Mitsui A, Nishiyama A, Nozaki K, Sono H, Gon Y, Hashimoto N, and Yodoi J. Overexpression of thioredoxin in transgenic mice attenuates focal ischemic brain damage. *Proc Natl Acad Sci U S A* 96: 4131–4136, 1999.
36. Takagi Y, Nozaki K, Sugino T, Hattori I, and Hashimoto N. Phosphorylation of c-Jun NH_2-terminal kinase and p38 mitogen-activated protein kinase after transient forebrain ischemia in mice. *Neurosci Lett* 294: 117–120, 2000.
37. Takagi Y, Hattori I, Nozaki K, Mitsui A, Ishikawa M, Hashimoto N, and Yodoi J. Excitotoxic hippocampal injury is attenuated in thioredoxin transgenic mice. *J Cereb Blood Flow Metab* 20: 829–833, 2000.
38. Takasago T, Peters EE, Graham DI, Masayasu H, and Macrae IM. Neuroprotective efficacy of ebselen, an antioxidant with anti-inflammatory actions, in a rodent model of permanent middle cerebral artery occlusion. *Br J Pharmacol* 122: 1251–1256, 1997.
39. Terada LS, Willingham IR, Rosandich ME, Leff JA, Kindt GW, and Repine JE. Generation of superoxide anion by brain endothelial cell xanthine oxidase. *J Cell Physiol* 148: 191–196, 1991.
40. Uyama O, Matsuyama T, Michishita H, Nakamura H, and Sugita M. Protective effects of human recombinant superoxide dismutase on transient ischemic injury of CA1 neurons in gerbils. *Stroke* 23: 75–81, 1992.
41. Van der Worp HB, Bar PR, Kappelle LJ, and Wildt DJ. Dietary vitamin E levels affect outcome of permanent focal cerebral ischemia in rats. *Stroke* 29: 1002–1006, 1998.
42. Weiss SJ. Tissue destruction by neutrophils. *N Engl J Med* 320: 365–376, 1989.
43. Zang RL, Chopp M, Jiang N, Tang WX, Prostak J, Manning AM, and Anderson DC. Anti-intracellular adhesion molecule-1 antibody reduces ischemic cell damage after transient but not permanent middle cerebral artery occlusion in the Wistar rat. *Stroke* 26: 1438–1443, 1995.

Address reprint requests to:
Junji Yodoi, M.D., Ph.D.
Department of Biological Responses
Institute for Virus Research
Kyoto University
53 Shogoin, Kawahara-cho
Sakyo-Ku, Kyoto, Japan 606-8507

E-mail: yodoi@ virus.kyoto-u.ac.jp

Received for publication June 13, 2003; accepted October 1, 2003.

This article has been cited by:

1. Meijuan Zhang, Chengrui An, Yanqin Gao, Rehana K. Leak, Jun Chen, Feng Zhang. 2012. Emerging roles of Nrf2 and phase II antioxidant enzymes in neuroprotection. *Progress in Neurobiology* . [CrossRef]
2. Gab Seok Kim, Joo Eun Jung, Purnima Narasimhan, Hiroyuki Sakata, Pak H. Chan. 2012. Induction of thioredoxin-interacting protein is mediated by oxidative stress, calcium, and glucose after brain injury in mice. *Neurobiology of Disease* **46**:2, 440-449. [CrossRef]
3. Ryusuke ONO, Taro MASAKI, Siphora DIEN, Xijun YU, Atsushi FUKUNAGA, Junji YODOI, Chikako NISHIGORI. 2012. Suppressive effect of recombinant human thioredoxin on ultraviolet light-induced inflammation and apoptosis in murine skin. *The Journal of Dermatology* no-no. [CrossRef]
4. Y.H. Ma, Ning Su, X.D. Chao, Y.Q. Zhang, Lei Zhang, Feng Han, Peng Luo, Zhou Fei, Yan Qu. 2012. Thioredoxin-1 attenuates post-ischemic neuronal apoptosis via reducing oxidative/nitrative stress. *Neurochemistry International* **60**:5, 475-483. [CrossRef]
5. Y.H. Ma, Ning Su, X.D. Chao, Y.Q. Zhang, Lei Zhang, Feng Han, Peng Luo, Zhou Fei, Yan Qu. 2012. Thioredoxin-1 attenuates post-ischemic neuronal apoptosis via reducing oxidative/nitrative stress. *Neurochemistry International* . [CrossRef]
6. Christine Lehner , Renate Gehwolf , Herbert Tempfer , Istvan Krizbai , Bernhard Hennig , Hans-Christian Bauer , Hannelore Bauer . Oxidative Stress and Blood–Brain Barrier Dysfunction Under Particular Consideration of Matrix Metalloproteinases. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
7. CONNIE H.Y. WONG, LATASHA D. ABEYNAIKE, PETER J. CRACK, MICHAEL J. HICKEY. 2011. Divergent Roles of Glutathione Peroxidase-1 (Gpx1) in Regulation of Leukocyte-Endothelial Cell Interactions in the Inflamed Cerebral Microvasculature. *Microcirculation* **18**:1, 12-23. [CrossRef]
8. Maria Laura Aon-Bertolino, Juan Ignacio Romero, Pablo Galeano, Mariana Holubiec, Maria Sol Badorrey, Gustavo Ezequiel Saraceno, Eva-Maria Hanschmann, Christopher Horst Lillig, Francisco Capani. 2011. Thioredoxin and glutaredoxin system proteins—immunolocalization in the rat central nervous system. *Biochimica et Biophysica Acta (BBA) - General Subjects* **1810**:1, 93-110. [CrossRef]
9. Yan Chen, Liwen Chang, Wenbin Li, Zhihui Rong, Wei Liu, Ruiyan Shan, Rui Pan. 2010. Thioredoxin protects fetal type II epithelial cells from hyperoxia-induced injury. *Pediatric Pulmonology* **45**:12, 1192-1200. [CrossRef]
10. Sergio Rosales-Corral , Russel J. Reiter , Dun-Xian Tan , Genaro G. Ortiz , Gabriela Lopez-Armas . 2010. Functional Aspects of Redox Control During Neuroinflammation. *Antioxidants & Redox Signaling* **13**:2, 193-247. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
11. Jin-Hee Sung, Eun-Hae Cho, Wongi Min, Mi-Jeong Kim, Myeong-Ok Kim, Eun-Jung Jung, Phil-Ok Koh. 2010. Identification of proteins regulated by estradiol in focal cerebral ischemic injury—A proteomics approach. *Neuroscience Letters* **477**:2, 66-71. [CrossRef]
12. Li Kong, Xiaohong Zhou, Feng Li, Juni Yodoi, James McGinnis, Wei Cao. 2010. Neuroprotective effect of overexpression of thioredoxin on photoreceptor degeneration in Tubby mice. *Neurobiology of Disease* **38**:3, 446-455. [CrossRef]
13. Guo-Hua Lin , Lin Lin , Hua-Wei Liang , Xin Ma , Jing-Ye Wang , Li-Ping Wu , Hui-Di Jiang , Iain C. Bruce , Qiang Xia . 2010. Antioxidant Action of a Chrysanthemum morifolium Extract Protects Rat Brain Against Ischemia and Reperfusion Injury. *Journal of Medicinal Food* **13**:2, 306-311. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
14. Phil-Ok KOH. 2010. Proteomic Analysis of Focal Cerebral Ischemic Injury in Male Rats. *Journal of Veterinary Medical Science* **72**:2, 181-185. [CrossRef]
15. Xiang Yang Zhang, Da Chun Chen, Mei Hong Xiu, Fan Wang, Ling Yan Qi, Hong Qiang Sun, Song Chen, Shu Chang He, Gui Ying Wu, Colin N. Haile. 2009. The novel oxidative stress marker

thioredoxin is increased in first-episode schizophrenic patients. *Schizophrenia Research* **113**:2-3, 151-157. [CrossRef]

16. Li-Na Sun, Jia Shen, Fang Su, Qian Wang, Yu-Jin Zhu, Xiao-E. Lou, Hua-Wei Liang, Iain C. Bruce, Qiang Xia. 2009. Bicyclol attenuates oxidative stress and neuronal damage following transient forebrain ischemia in mouse cortex and hippocampus. *Neuroscience Letters* **459**:2, 84-87. [CrossRef]
17. N. Kobayashi, Y. Yamada, W. Ito, S. Ueki, H. Kayaba, H. Nakamura, J. Yodoi, J. Chihara. 2009. Thioredoxin reduces C-C chemokine-induced chemotaxis of human eosinophils. *Allergy* **64**:8, 1130-1135. [CrossRef]
18. Stefan Hofer, Claudia Rosenhagen, Hajime Nakamura, Junji Yodoi, Christian Bopp, Johannes B. Zimmermann, Meike Goebel, Peter Schemmer, Kartrin Hoffmann, Klaus Schulze-Osthoff, Raoul Breitkreutz, Markus A. Weigand. 2009. Thioredoxin in human and experimental sepsis*. *Critical Care Medicine* **37**:7, 2155-2159. [CrossRef]
19. Jguirim-Souissi Imen, Ludivine Billiet, Clarisse Cuaz-Pérolin, Nadège Michaud, Mustapha Rouis. 2009. The regulated in development and DNA damage response 2 (REDD2) gene mediates human monocyte cell death through a reduction in thioredoxin-1 expression. *Free Radical Biology and Medicine* **46**:10, 1404-1410. [CrossRef]
20. Jin-Hee Sung, Eun-Hae Cho, Myeong-Ok Kim, Phil-Ok Koh. 2009. Identification of proteins differentially expressed by melatonin treatment in cerebral ischemic injury - a proteomics approach. *Journal of Pineal Research* **46**:3, 300-306. [CrossRef]
21. Parameswaran G. Sreekumar, Yi Ding, Stephen J. Ryan, Ram Kannan, David R. Hinton. 2009. Regulation of thioredoxin by ceramide in retinal pigment epithelial cells#. *Experimental Eye Research* **88**:3, 410-417. [CrossRef]
22. Jie Jia, Xi Zhang, Yong-Shan Hu, Yi Wu, Qing-Zhi Wang, Na-Na Li, Cai-Qin Wu, Hui-Xian Yu, Qing-Chuan Guo. 2009. Protective effect of tetraethyl pyrazine against focal cerebral ischemia/reperfusion injury in rats: Therapeutic time window and its mechanism. *Thrombosis Research* **123**:5, 727-730. [CrossRef]
23. E. V. Kalinina, N. N. Chernov, A. N. Saprin. 2008. Involvement of thio-, peroxi-, and glutaredoxins in cellular redox-dependent processes. *Biochemistry (Moscow)* **73**:13, 1493-1510. [CrossRef]
24. N MAULIK, D DAS. 2008. Emerging potential of thioredoxin and thioredoxin interacting proteins in various disease conditions. *Biochimica et Biophysica Acta (BBA) - General Subjects* **1780**:11, 1368-1382. [CrossRef]
25. H HU, L LU, W MU, R JOHNSON, E BLOCK, J PATEL. 2008. Priming Donor Lungs With Thioredoxin-1 Attenuates Acute Allograft Injury in a Rat Model of Lung Transplantation. *The Journal of Heart and Lung Transplantation* **27**:10, 1142-1149. [CrossRef]
26. Yuma Hoshino , Keisuke Shioji , Hajime Nakamura , Hiroshi Masutani , Junji Yodoi . 2007. From Oxygen Sensing to Heart Failure: Role of Thioredoxin. *Antioxidants & Redox Signaling* **9**:6, 689-699. [Abstract] [Full Text PDF] [Full Text PDF with Links]
27. Keiichiro Sakuma, Hajime Nakamura, Takayuki Nakamura, Yuma Hoshino, Shugo Ueda, Masataka Ichikawa, Chiharu Tabata, Shiro Fujita, Katsuhiro Masago, Junji Yodoi, Michiaki Mishima, Tadashi Mio. 2007. Elevation of Serum Thioredoxin in Patients with Gefitinib-induced Interstitial Lung Disease. *Internal Medicine* **46**:23, 1905-1909. [CrossRef]
28. Hiroyuki Tamaki, Hajime Nakamura, Akiyoshi Nishio, Hiroshi Nakase, Satoru Ueno, Norimitsu Uza, Masahiro Kido, Satoko Inoue, Sakae Mikami, Masanori Asada, Keiichi Kiriya, Hiroshi Kitamura, Shinya Ohashi, Toshiro Fukui, Kimio Kawasaki, Minoru Matsuura, Yasuyuki Ishii, Kazuichi Okazaki, Junji Yodoi, Tsutomu Chiba. 2006. Human Thioredoxin-1 Ameliorates Experimental Murine Colitis in Association With Suppressed Macrophage Inhibitory Factor Production. *Gastroenterology* **131**:4, 1110-1121. [CrossRef]

29. L Tao, E Gao, A Hu, C Coletti, Y Wang, T A Christopher, B L Lopez, W Koch, X L Ma. 2006. Thioredoxin reduces post-ischemic myocardial apoptosis by reducing oxidative/nitrative stress. *British Journal of Pharmacology* **149**:3, 311-318. [CrossRef]
30. Atsuyasu Sato , Tomijiro Hara , Hajime Nakamura , Noriko Kato , Yuma Hoshino , Norihiko Kondo , Michiaki Mishima , Dr. Junji Yodoi . 2006. Thioredoxin-1 Suppresses Systemic Inflammatory Responses Against Cigarette Smoking. *Antioxidants & Redox Signaling* **8**:9-10, 1891-1896. [Abstract] [Full Text PDF] [Full Text PDF with Links]
31. Tsuyoshi Ohta, Ken-ichiro Kikuta, Hirotoshi Imamura, Yasushi Takagi, Masaki Nishimura, Yoshiki Arakawa, Nobuo Hashimoto, Kazuhiko Nozaki. 2006. Administration of Ex Vivo-expanded Bone Marrow-derived Endothelial Progenitor Cells Attenuates Focal Cerebral Ischemia-reperfusion Injury in Rats. *Neurosurgery* **59**:3, 679-686. [CrossRef]
32. S UEDA, T NAKAMURA, A YAMADA, A TERATANI, N MATSUI, S FURUKAWA, Y HOSHINO, M NARITA, J YODOI, H NAKAMURA. 2006. Recombinant human thioredoxin suppresses lipopolysaccharide-induced bronchoalveolar neutrophil infiltration in rat. *Life Sciences* **79**:12, 1170-1177. [CrossRef]
33. Yasuya Inomata, Hajime Nakamura, Masaki Tanito, Akie Teratani, Takahiro Kawaji, Norihiko Kondo, Junji Yodoi, Hidenobu Tanihara. 2006. Thioredoxin inhibits NMDA-induced neurotoxicity in the rat retina. *Journal of Neurochemistry* **98**:2, 372-385. [CrossRef]
34. Junya Hayashi, Yasushi Takagi, Hitoshi Fukuda, Takayuki Imazato, Masaki Nishimura, Motoaki Fujimoto, Jun Takahashi, Nobuo Hashimoto, Kazuhiko Nozaki. 2006. Primate embryonic stem cell-derived neuronal progenitors transplanted into ischemic brain. *Journal of Cerebral Blood Flow & Metabolism* **26**:7, 906-914. [CrossRef]
35. Kumuda C. Das . 2005. Thioredoxin and Its Role in Premature Newborn Biology. *Antioxidants & Redox Signaling* **7**:11-12, 1740-1743. [Abstract] [Full Text PDF] [Full Text PDF with Links]
36. Anne Burke-Gaffney, Matthew E.J. Callister, Hajime Nakamura. 2005. Thioredoxin: friend or foe in human disease?. *Trends in Pharmacological Sciences* **26**:8, 398-404. [CrossRef]
37. Hee Sun Byun, Eun Wie Cho, Jin Sik Kim, Myung Sook Moon, Jung Joo Yum, Kug Chan Kim, In Gyu Kim. 2005. Thioredoxin overexpression in HT-1080 cells induced cellular senescence and sensitization to gamma radiation. *FEBS Letters* **579**:19, 4055-4062. [CrossRef]
38. Hajime Nakamura . 2005. Thioredoxin and Its Related Molecules: Update 2005. *Antioxidants & Redox Signaling* **7**:5-6, 823-828. [Abstract] [Full Text PDF] [Full Text PDF with Links]
39. Songlin Li, Jian Zheng, S. Thomas Carmichael. 2005. Increased oxidative protein and DNA damage but decreased stress response in the aged brain following experimental stroke. *Neurobiology of Disease* **18**:3, 432-440. [CrossRef]
40. Takayuki Nakamura , Hajime Nakamura , Tomoaki Hoshino , Shugo Ueda , Hiromi Wada , Junji Yodoi . 2005. Redox Regulation of Lung Inflammation by Thioredoxin. *Antioxidants & Redox Signaling* **7**:1-2, 60-71. [Abstract] [Full Text PDF] [Full Text PDF with Links]
41. S ANDRABI. 2004. Oxyresveratrol (trans-2,3?,4,5?-tetrahydroxystilbene) is neuroprotective and inhibits the apoptotic cell death in transient cerebral ischemia. *Brain Research* **1017**:1-2, 98-107. [CrossRef]
42. Hajime Nakamura . 2004. Thioredoxin as a Key Molecule in Redox Signaling. *Antioxidants & Redox Signaling* **6**:1, 15-17. [Citation] [Full Text PDF] [Full Text PDF with Links]