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Thioredoxin-1 attenuates indomethacin-induced gastric mucosal injury in mice

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Abstract
Indomethacin is one of non-steroidal anti-inflammatory drugs that are commonly used clinically and often cause gastric mucosal injury as a side effect. Generation of reactive oxygen species (ROS) and activation of apoptotic signaling are involved in the pathogenesis of indomethacin-induced gastric mucosal injury. Thioredoxin-1 (Trx-1) is a small redox-active protein with anti-oxidative activity and redox-regulating functions. The aim of this study was to investigate the protective effect of Trx-1 against indomethacin-induced gastric mucosal injury. Trx-1 transgenic mice displayed less gastric mucosal damage than wild type (WT) C57BL/6 mice after intraperitoneal administration of indomethacin. Administration of recombinant human Trx-1 (rhTrx-1) or transfection of the Trx-1 gene reduced indomethacin-induced cytotoxicity in rat gastric epithelial RGM-1 cells. Pretreatment with rhTrx-1 suppressed indomethacininduced ROS production and downregulation of phosphorylated Akt in RGM-1 cells. Survivin, a member of inhibitors of apoptosis proteins family, was downregulated by indomethacin, which was suppressed in Trx-1 transgenic mice or by administration of rhTrx-1 in RGM-1 cells. Trx-1 inhibits indomethacin-induced apoptotic signaling and gastric ulcer formation, suggesting that it may have a preventive and therapeutic potential against indomethacin-induced gastric injury.

Keywords: *Thioredoxin, redox, indomethacin, survivin, apoptosis*

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin are the most commonly prescribed drugs for inflammation, arthritis, and cardiovascular protection. It is generally accepted that the primary mechanism by which these agents exert their anti-inflammatory action is through inhibiting the synthesis of prostaglandins (PGs), especially PGE2, by blocking the cyclooxygenase (COX) isoenzymes, COX-1, and COX-2. However, this mechanism of action by NSAIDs is often associated with a significant risk of hemorrhage, erosion, and perforation of gastric ulcers [1]. Reactive oxygen species (ROS) are involved in indomethacin-induced gastric injury, and certain compounds with antioxidative activity reduce its incidence and severity [2–4]. Survivin, a 16.5-kDa protein expressed in the surface epithelial cells and neck cells of both human and rat gastric mucosa, is a member of inhibitors of

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apoptosis proteins (IAPs) family and a broad-spectrum suppressor of cell death [5]. A recent study has shown that indomethacin causes severe injury to gastric mucosa and rat gastric epithelial RGM-1 cells by reducing survivin levels, since this protein plays a role in gastric mucosal integrity and the protection of the gastric mucosa against injury [6,7]. Thus, scavenging ROS and modulating survivin levels could reduce indomethacininduced gastric injury.

Thioredoxin-1 (Trx-1) is a small ubiquitous protein with a redox-active disulfide/dithiol within the conserved active site sequence, –Cys–Gly–Pro–Cys– [8,9]. Reduced Trx-1, which has dithiols in its active site, can function as a general protein disulfide reductase and oxidized Trx-1, which has a disulfide bond in its active site, is reduced by NADPH and Trx reductase. Human Trx-1, which was originally cloned as adult T-cell leukemia-derived factor (ADF) produced by human T-cell leukemia virus type I-transformed T-cells [10], possesses multiple biological functions and regulates a variety of systems via thiol-dependent redox control. Trx-1 is induced by a variety of stresses including viral infection and hydrogen peroxide [11,12] and is involved in the redox-regulation of signal transduction [13,14]. It scavenges ROS such as singlet oxygen, hydroxyl radical, and H_2O_2 [15,16]. Intracellular Trx-1 inhibits the activation of apoptosis signal-regulating kinase-1 (ASK-1) [17] and p38 mitogenactivated protein kinase (MAPK) [18]. Exogenous administration of recombinant human Trx-1 (rhTrx-1) can suppress hydrogen peroxide-induced cytotoxicity *in vitro* [19] and 1-methyl-4-phenylpyridinium-induced neurotoxicity in the rat [20], brain damage following transient focal cerebral ischemia in mice [21], proinflammatory cytokine- or bleomycin-induced lung injury [22], and ethanol-induced injury of gastric mucosal cells [23,24]. Trx-1 transgenic mice, in which human Trx-1 is systemically overexpressed under the control of the β-actin promoter, have increased resistance to focal cerebral ischemia [18], retinal photo-oxidative damage [25], and renal ischemia/reperfusion injury [26]. It remains unclear, however, whether Trx-1 is involved in the mechanism of protection against indomethacin-induced gastric mucosal injury.

In this study, we demonstrate that Trx-1 transgenic mice are more resistant to the formation of indomethacin-induced gastric ulcers than wild type (WT) C57BL/6 mice. We used rat gastric epithelial RGM-1 cells to investigate the molecular basis of this protective effect. Treatment of RGM-1 cells with human recombinant Trx-1 (rhTrx-1) inhibited indomethacin-induced ROS generation and cytotoxicity. Moreover, rhTrx-1 inhibited the indomethacin-induced apoptotic signals by preserving phosphorylated Akt and survivin. These observations suggest that Trx-1 plays a crucial role in scavenging ROS and modulating apoptotic signaling and may have a good clinical potential against indomethacin-induced gastric injury.

Materials and methods

Reagents

DMEM/Ham's F12 medium (1: 1 v/v) was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Indomethacin was from Sigma Chemical Co. (St Louis, MO), and 2′, 7′-dichlorofluorescein diacetate (DCFH-DA) was from molecular probes (Eugene, OR, USA). Cytotoxicity Detection Kits (LDH release assay) were purchased from Roche Diagnostics GmbH (Germany). Endotoxin-free rhTrx-1 was prepared as described previously [16] and provided by Ajinomoto, Inc. (Kawasaki, Japan). Anti-human Trx-1 antibody was obtained from Redox Bioscience, (Kyoto, Japan). Anti-mouse Trx-1 antibody which was proved to cross-react with rat Trx was prepared as previously described [27]. Anti-phospho-Akt (Ser 473) and anti-Akt rabbit polyclonal antibodies were purchased from Cell Signaling Technology, Inc., Beverly, MA and antisurvivin rabbit polyclonal antibody was purchased from Santa Cruz Biotechnology Inc., Santa Cruz, CA.

Animals

Age- and sex-matched C57BL/6 mice were purchased from Clea (Tokyo, Japan). Trx-1-transgenic (Trx-1 Tg) mice were generated from C57BL/6 mice using a transgene composed of the β-actin promoter and human Trx-1 gene, as described previously [18]. In these Trx-1 Tg mice, the expression level of human Trx-1 in each tissue including the stomach is in 3-5 fold higher than endogenous murine Trx-1 level [18]. The mice were housed at a constant temperature and supplied with laboratory chow and water *ad libitum*. The Animal Research Committee of the Institute for Virus Research, Kyoto University, approved all of the animal experiments reported in this paper.

Cell culture

Rat gastric epithelial cell line RGM-1 (RIKEN Cell Bank, Japan) was cultured in Dulbecco's Modified Eagle's Medium (DMEM)/Ham's F12 medium (Nacalai Tesque, Japan) supplemented with 10% fetal calf serum, 100 μg/ml streptomycin and 100 U/ml penicillin in a humidified atmosphere under 5% CO_2 at 37°C.

Indomethacin-induced gastric injury model

C57BL/6 WT and Trx-1 Tg mice (8 weeks old, weighing 19–22 g) were fasted for about 16 h. Three

mice per group were treated intraperitoneally with either 40 mg/kg body weight indomethacin or vehicle (5% sodium bicarbonate). After the mice were killed, their stomachs were removed, opened along the greater curve, and washed with physiological saline. Six hours after indomethacin treatment, gastric mucosal specimens were collected for Western blot analysis of p-Akt and survivin. Twenty-four hours after indomethacin treatment, the stomachs were flattened and observed using surgical microscopy. Images of the fresh glandular stomachs were digitalized and their surface areas were calculated with a computer-assisted analysis system. The initial assessment involved determining the percentage of gastric mucosal damage, which was calculated as follows:

$$\%\,\text{Gastric mucosal damage} = (\text{total lesion area}/\text{total area of the gastric mucosa}) \times 100$$

Histological analysis of indomethacin-induced gastric damage

After fixation with 10% neutral buffered formalin and embedding in paraffin, the stomachs were cut into 4-μm thick sections at 1-mm horizontal intervals from the fundus to the antrum, perpendicular to the mucosal surface, and the sections were stained with hematoxylin–eosin (HE). Two experienced investigators, who were blind to the genotypes of the mice, performed histological examinations of coded slides. Damage to the epithelial surface was defined as the presence of ulcers, ranging from epithelial disruption to deep ulceration to the lamina propria in association with the inflammatory infiltrate. The assessment involved counting the number of ulcers in each section and determining gastric mucosal thickness with NIH 1.62 image software. Results were expressed as means ± standard deviation (SD).

Transfection

The wtTrx-1 (wild type) or dmTrx-1 (C32S/C35S double mutant)/p3 × Flag-CMV-10 plasmids were constructed as described previously [28]. dmTrx-1 shows no reducing activity in which two cysteines in the active site are replaced with serines. RGM-1 cells were transfected with Trx-1-wt or Trx-1 dm/ p3 × Flag-CMV-10 vector using TransIT®-LT1 reagent (Mirus Bio Corporation, Madison, WI) according to the manufacturer's instructions. After cultured with fresh medium for 48 h, the transiently transfected cells were used for subsequent experiments.

Western blotting

Lysates of gastric tissues and RGM-1 cells were obtained by incubating with lysis buffer (20 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, 1 mM Na_3VO_4, and 1 μg/ml leupeptin) for 30 min on ice. They were cleared by centrifugation and kept at 95°C for 5 min with SDS sample buffer. They were then subjected to 10% (for Akt and p-Akt) or 15% (for Trx and Survivin) SDS-PAGE, and transferred to PDVF membranes (Millipore, Bedford, MA). After blocking with 5% skim milk in TBS containing 0.05% Tween-20 for 2 h at room temperature, the membranes were incubated for 2 h with primary antibodies at room temperature or overnight at 4°C followed by peroxidase-conjugated anti-rabbit or anti-mouse IgG (Amersham Biosciences, Tokyo, Japan) for 1 h at room temperature. An ECL Western blot detection kit (Amersham Biosciences, Tokyo, Japan) was used to visualize the epitopes.

Measurement of intracellular ROS by flow cytometry

Cells were cultured in 100 mm dishes (2×10^5) overnight and changed to serum-free Dulbecco's Modified Eagle's Medium (DMEM)/Ham's F12 medium. The cells were pretreated with 100 μg/ml rhTrx for 1 h and then with indomethacin (200 μM) for 12 h at 37°C. To examine the effects of Trx-1 on the production of ROS, the cells were incubated with 5 μM DCFH-DA in the dark for the last 30 min of the treatment. They were washed once with PBS, harvested by trypsinization, and washed twice more before being suspended in FACS solution. 2′,7′-Dicholorfluorescein (DCF) fluorescence resulting from the oxidation of DCFH-DA was measured in samples of 10,000 cells with FACSCalibur (Nippon Becton Dickinson, Tokyo, Japan) as described previously [29].

Lactate dehydrogenase (LDH) release assay

Cell viability was assessed by the LDH release assay. LDH released from damaged cells was determined in aliquots of the culture medium according to the manufacturer's procedure. The percentage cytotoxicity was calculated by the following formula supplied by the manufacturer.

$$\text{Cytotoxicity}(\%) = \frac{[(\text{Experimental LDH release } (OD_{492}) - \text{Spontaneous LDH release } (OD_{492}) \times 100}{\text{Maximum LDH release } (OD_{492}) - \text{Spontaneous LDH release } (OD_{492})}$$

The OD_{492} of spontaneous LDH release was obtained from the culture supernatant of vehicle-treated controls, and the OD492 of maximum LDH release was from the positive control (cells treated with 2% Triton X-100).

Determination of PGE2 production in the indomethacin-treated gastric mucosa

C57BL/6 WT or Trx-1 Tg mice (each group, $n = 3$) were injected intraperitoneally with either 40 mg/kg body weight of indomethacin or vehicle (5% sodium bicarbonate) and left for 6, 12, or 24 h. PGE2 production in the gastric mucosa was assessed by the previously described method [30] with some modification. Gastric mucosal samples were minced in Tris–HCl buffer (150 mmol/l, pH 8.2) for 1 min and centrifuged at 9000 rpm for 1 min. The supernatants were discarded, 1.5 ml of fresh Tris–HCl buffer was added, and the specimens were vortexed for 1 min, followed by addition of 10 mmol/l indomethacin to terminate PGE2 production. They were then centrifuged for 1 min at 9000 rpm, and the amount of PGE2 was determined by enzyme immunoassay using the PGE2 Biotrak Enzyme Immunoassay (EIA) system (Amersham Biosciences).

After measuring the protein concentration of the tissue specimens, PGE2 production (ng/mg tissue/min) was determined for each sample.

Statistical analysis

The results are expressed as means ± SD, unless otherwise stated. The statistical significance of differences was evaluated with Student's *t*-test; $p < 0.05$ was considered statistically significant.

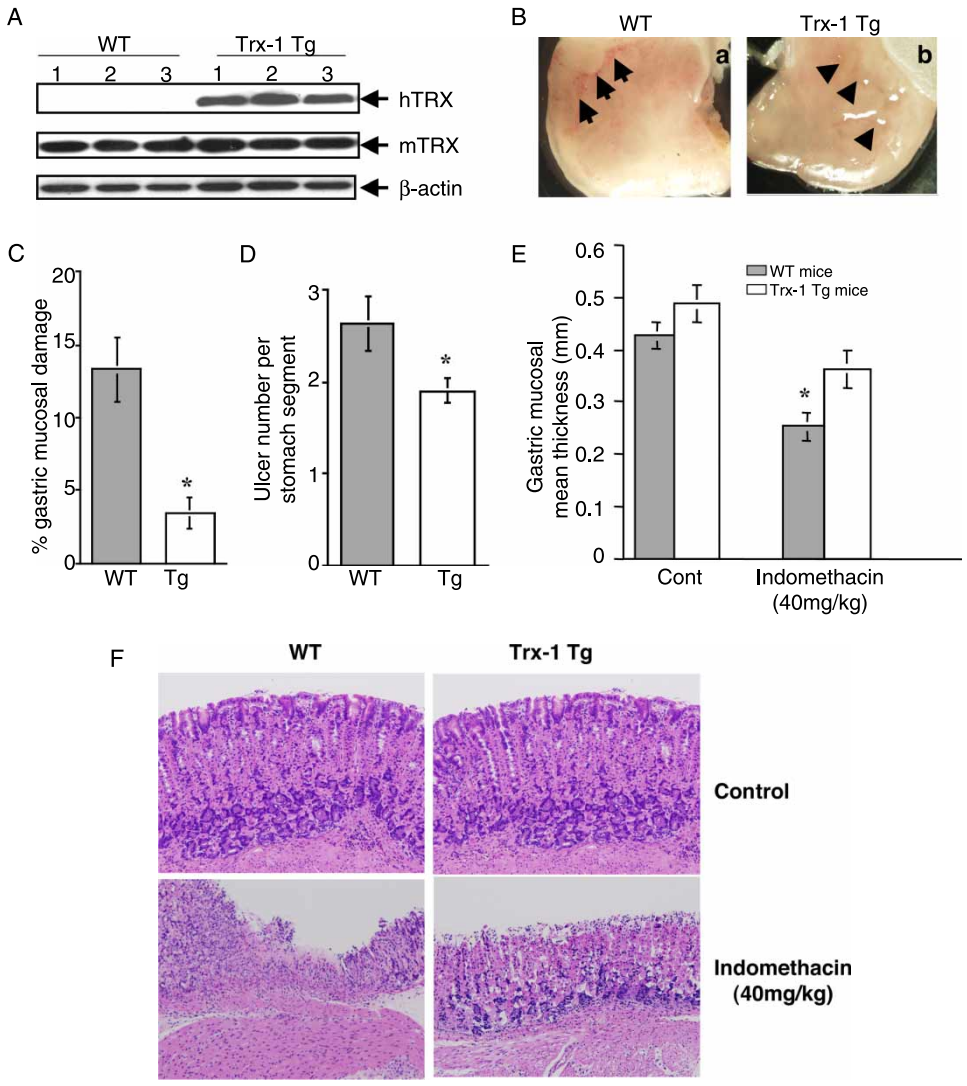

Figure 1. Macroscopic and histological assessment of gastric mucosal damage induced by indomethacin. (A) Western blot analysis of exogenous human Trx-1 and endogenous murine Trx-1 expression in the stomachs of WT and Trx-1 Tg mice. (B) Macroscopic appearance of the stomachs: (a) extensive hemorrhage mucosal lesions in the stomachs of WT mice; and (b) small hemorrhagic lesions in the stomachs of Trx-1 Tg mice. (C) The level of gastric mucosal damage in Trx-1 Tg mice was significantly lower than in WT mice ($*p < 0.001$). (D) The mean number of ulcers seen in Trx-1 Tg mice is lower than in WT mice ($*p < 0.05$). (E) The mean thickness of the gastric mucosa in WT mice is lower than in Trx-1-Tg mice ($*p < 0.05$). (F) Representative histological appearance of the stomachs of indomethacin-administered WT and Trx-1 Tg mice (HE: × 100). Upper panels represent the normal mucosa of WT mice and Trx-1 Tg mice respectively. Lower panels show the typical lesions induced by indomethacin.

Results

Increased resistance to indomethacin-induced gastric mucosal damage in Trx-1 transgenic mice

The expression of exogenous human Trx-1 protein in the stomachs of Trx-1 Tg mice was confirmed by Western blotting (Figure 1(A)). Observation with a surgical microscope showed that the extent and severity of indomethacin-induced gastric damage in the Trx-1 Tg mice was less than that in WT mice. Extensive hemorrhagic mucosal lesions were seen in the WT mice, while these lesions were present in much smaller size in the Trx-1 Tg mice (Figure 1(B)). Histological examination of traversing sections of the stomach showed that the level of gastric mucosal damage induced by indomethacin in the Trx-1 Tg mice was lower than that in WT mice (Figure 1(C)). The typical lesion observed in the indomethacin-treated mucosa of WT mice exhibited broad-based gastric erosion with major loss of surface and mucous neck cells and loss of integrity of the glands. In the indomethacin-treated mucosa of Trx-1 Tg mice, lesions were less apparent, although the apical regions of some of the glands were disorganized and there was some general loss of surface mucous and mucous neck cells (Figure 1(D)). There were significant differences in the number of ulcers (Figure 1(E)) and the gastric mucosal mean thickness (Figure 1(F)) between Trx-1 Tg and WT mice. It can be concluded that the over-expression of Trx-1 in Trx-1 Tg mice confers resistance to indomethacin-induced gastric injury.

Effect of rhTrx-1 and overexpression of Trx-1 on the cytotoxic effect of indomethacin in RGM-1 cells

LDH analysis showed that the cytotoxicity increased by the treatment with indomethacin in a dose-dependent manner (Figure 2(A)). When RGM-1 cells were treated with indomethacin after the pretreatment with different concentrations of rhTrx-1, we observed a dose-dependent suppression of the indomethacin-induced cytotoxicity by rhTrx-1 (Figure 2(B)). Transfection of RGM-1 cells with p3 × Flag-tagged-wtTrx-1, p3 × Flag tagged-dmTrx-1 and control vector showed that transient overexpression of wtTrx-1 but not dmTrx-1 inhibited the indomethacin-induced cytotoxicity (Figure 2(C)).

Inhibition of PGE_2 production in indomethacin-treated wild type and hTrx-1-transgenic mice

We examined COX activity in the gastric mucosa of WT mice and Trx-1 Tg mice by measuring PGE2 production. Indomethacin (40 mg/kg body weight) inhibited PGE2 production not only in WT mice but also in Trx-1 Tg mice at 6, 12, and 24 h (Figure 3). It suggested that the protective effect of Trx-1 was independent of PGE2 production.

Pretreatment with rhTrx-1 suppresses indomethacin-induced ROS formation in rat gastric mucosal RGM-1 cells

To address the molecular mechanism of the protective effect of Trx-1 against indomethacin-induced gastric injury, we analyzed ROS in rat gastric mucosal RGM-1 cells after the treatment with indomethacin. Flow cytometric measurements of intracellular ROS using DCFA-DA showed that the treatment with indomethacin (200 μM) for 12 h led to increase intracellular production of ROS production in RGM-1 cells, which was suppressed by 1 h pretreatment with rhTrx-1 (100 μg/ml) (Figure 4).

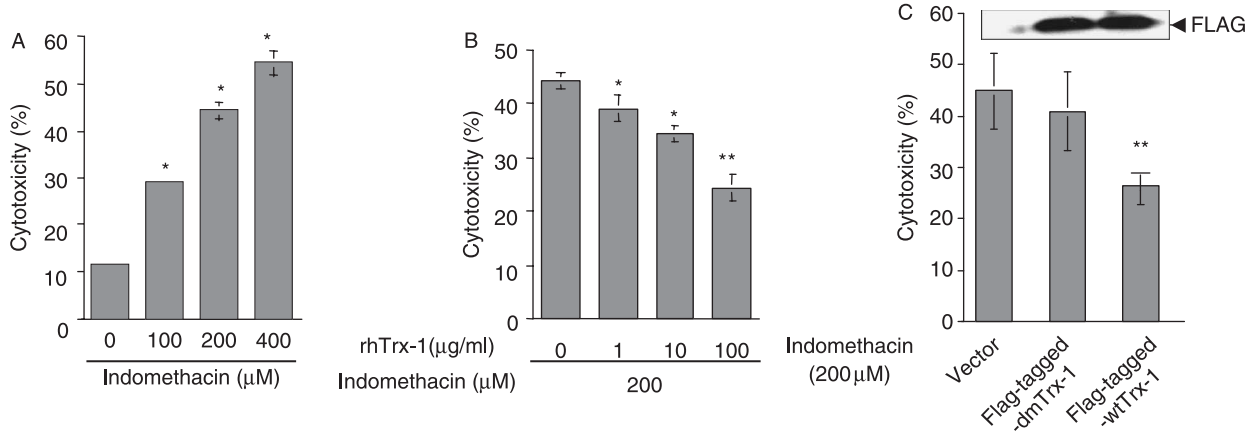

Figure 2. Administration of rhTrx-1 or overexpression of hTrx-1 suppresses indomethacin-induced injury. (A) The cytotoxicity was determined by LDH release from RGM-1 cells after treatment with 0, 100, 200, and 400 μM indomethacin for 24 h. (B) LDH release from RGM-1 cells by 24 h treatment with 200 μM indomethacin after 1 h preincubation with 0, 1, 10, 100 μg/ml rhTrx-1. (C) LDH release was determined after 200 μM indomethacin treatment from RGM-1 cells transfected with control vector, p3 × Flag-tagged-wtTrx-1, or p3 × Flag tagged-dmTrx-1. Flag expression was checked by Western-blotting analysis. Experiments were performed in triplicate. Each column is expressed as means ± SD. Asterisks indicate statistical significance (*$p < 0.05$, **$p < 0.001$).

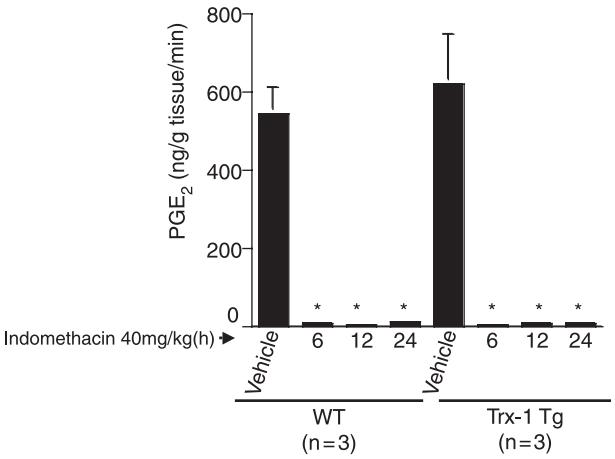

Figure 3. Inhibition of PGE2 production in indomethacin-treated WT mice and Trx-1 Tg mice. Mice in each group ($n = 3$) were treated with either 40 mg/kg body weight indomethacin or vehicle (5% sodium bicarbonate) intraperitoneally for 6, 12, or 24 h, respectively. PGE2 production by gastric tissue was determined by enzyme immunoassay. Each column represents means ± SD. $^{\star}p < 0.001$ vs. vehicle-treated mice; ($n = 3$).

Indomethacin-induced downregulation of Akt phosphorylation is suppressed in hTrx-1-transgenic mice and by pretreatment with rhTrx-1 in RGM-1 cells

Indomethacin treatment caused downregulation of phosphorylated-Akt, the active form of Akt, in the gastric mucosa of WT mice, while this effect was inhibited in the Trx-1 Tg mice (Figure 5(A)). In RGM-1 cells, pretreatment with rhTrx-1 (100 μg/ml) suppressed the indomethacin-induced downregulation of phosphorylated Akt (Figure 5(B)).

Indomethacin-induced downregulation of survivin is suppressed in hTrx-1-transgenic mice and by pretreatment with rhTrx-1 in RGM-1 cells

To investigate whether Trx-1 exerts its protective effect by affecting the anti-apoptotic survivin signaling pathway, we measured survivin levels in the gastric mucosa by Western blotting. Indomethacin reduced survivin levels in the gastric mucosa of WT mice, while this reduction was inhibited in the Trx-1 Tg mice (Figure 6(A)). Moreover, in RGM-1 cells, pretreatment with rhTrx-1 inhibited the indomethacin-induced downregulation of survivin (Figure 6(B)).

Discussion

We have shown previously that Trx-1 transgenic mice, in which human Trx-1 is overexpressed systemically under the control of the β-actin promoter, are relatively resistant to a variety of stress-associated disorders and survive longer than WT mice [18,22,31,32]. Trx-1 plays crucial roles *in vivo* as an antioxidant and a redoxregulating signaling molecule. The present study shows that indomethacin-induced gastric mucosal injury is dramatically attenuated in Trx-1-Tg mice (Figure 1). This is in line with our previous report that *Helicobacter felis*-induced gastritis is suppressed in Trx-1-Tg mice [33]. In the present study, we further analyzed the molecular mechanisms of the protective effect of Trx-1 against indomethacin-induced gastric mucosal injury using Trx-1 transgenic mice and RGM-1 cells.

In the stomach, PGs, especially PGE2, play an important role in maintaining gastric mucosal integrity via several mechanisms including regulation of gastric mucosal blood flow, turnover of epithelial cells, synthesis of mucus, and inhibition of gastric acid secretion [34]. Indomethacin is a NSAID that can suppress the production of PGs by inhibiting both COX1 and COX2. This in turn can lead to gastric mucosal damage as a side effect. In the present study, we observed that PGE2 production was inhibited by indomethacin in Trx-1-Tg as well as WT mice (Figure 3), suggesting that the protective function of Trx-1 against indomethacin-induced

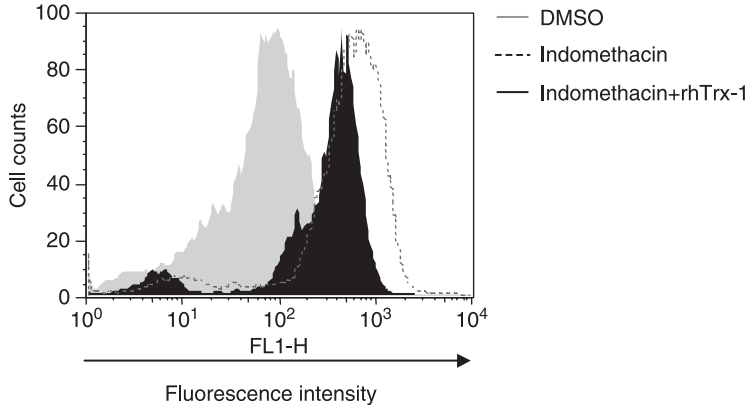

Figure 4. Suppression of indomethacin-induced generation of ROS in rhTrx-1 pretreated RGM-1 cells. RGM-1 cells (2×10^5) grown overnight were preincubated in 100 μg/ml rhTrx-1 for 1 h, then incubated with indomethacin (200 μM) in the absence of serum for 12 h at 37°C with 5% CO2. DCFH-DA (5 μM) was added for the last 30 min. Fluorescence intensity was measured by flow cytometry. Experiments were performed in triplicate.

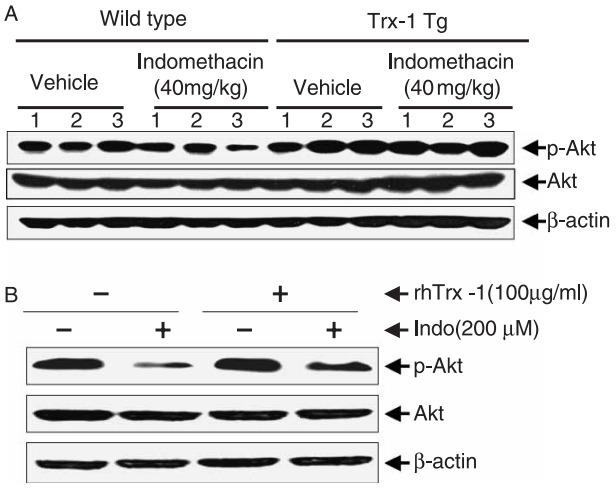

Figure 5. Trx-1 prevents indomethacin-induced downregulation of Akt phosphorylation in the gastric mucosa of Trx-1 Tg mice and rat gastric mucosal RGM-1 cells. (A) Total and phosphorylated Akt were detected in the gastric mucosa of Trx-1 Tg and WT mice after treatment with indomethacin (40 mg/kg) or vehicle for 6 h ($n = 3$ in each group). β-actin was demonstrated as a protein standard. (B) RGM-1 cells were cultured with rhTrx-1 (100 μg/ml) for 1 h and then treated with indomethacin (200 μM) for 24 h. Experiments were performed in triplicate.

gastric mucosal damage is independent of the COX pathway.

It was reported that indomethacin has pro-oxidant activity and initiates lipid peroxidation by generation of ROS, which play a crucial role in the gastric mucosal injury [35]. Previous reports showed that Trx-1 has radical scavenging activity against singlet oxygens and hydroxyl radicals [16]. Exogenous rhTrx-1 might interact with membrane-bound Trx itself or a member of Trx family on the cell surface or enter the cells [28] via lipid rafts to suppress intracellular

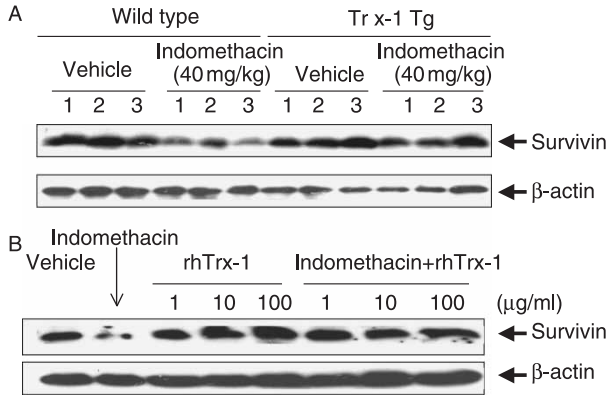

Figure 6. Effect of Trx-1 on survivin expression *in vivo* and *in vitro*. (A) Survivin protein expression in the gastric mucosa of Trx-1 Tg and WT mice after treatment with indomethacin (40 mg/kg) or vehicle for 6 h ($n = 3$ in each group). (B) RGM-1 cells were preincubated with 1, 10, and 100 μg/ml rhTrx-1 (Ajinomoto) for 1 h, then incubated with indomethacin (200 μM) in the absence of serum for 6 h. Experiments were performed in triplicate.

ROS (Kondo et al., unpublished data). In our present study, we demonstrated that pretreatment with rhTrx-1 suppressed the indomethacin-induced generation of ROS in RGM-1 cells (Figure 4). Therefore, the suppression of indomethacin-induced gastric mucosal injury in Trx-1-Tg mice may be partly due to suppression of ROS by Trx-1. Nitric oxide and inducible nitric oxide synthetase are also involved in the cytoprotection against indomethcin-induced gastric mucosal injury [36,37]. The relationship between Trx-1 and nitric oxide is to be clarified.

In addition to the antioxidant effect of Trx-1, administration of rhTrx-1 suppresses oxidative stress-induced apoptosis [28]. Akt is a crucial signaling molecule with effects on cell survival and apoptosis. Akt has a redox-sensitive intramolecular disulfide bond between its Cys-297 and Cys-311 and is redox-regulated. Hydrogen peroxide induces disulfide formation and inactivates Akt, and glutaredoxin, a redoxregulating protein, protects Akt from inactivation [38]. In our study, overexpression of Trx-1 in Trx-1 Tg mice or pretreatment with rhTrx-1 in RGM-1 cells preserved the phosphrylation of Akt, indicating that Trx-1 inhibited the indomethacin-induced inactivation of Akt (Figure 5(A),(B)). This result suggests that Trx-1 shows the protective effect against indomethacin-induced cytotoxicity at least partly through the preservation of the Akt-dependent anti-apoptotic signal.

Survivin, which belongs to the IAP family, protects cells from apoptosis [5]. Previous work showed that indomethacin reduces survivin expression in gastric mucosal cells, independently of COX activity [7]. Substantial reduction of surviving precedes severe gastric injury [7]. Deletion of the phosphatase and tensin homolog on chromosome ten (PTEN), a 54-kDa protein with dual specificity tyrosine/serine/threonine and lipid phosphatase activity, may interfere with apoptosis and cell proliferation by promoting survivin expression. This mechanism has been demonstrated in patients with endometrial carcinomas, in which survivin expression is correlated with decreased PTEN expression and increased phospho-Akt (p-Akt) [39]. PTEN exerts a major effect as a negative regulator of Akt activation via its phosphatidylinositol-3-phosphatase activity [40]. Trx-1 binds to and inhibits the lipid phosphatase activity of PTEN, up-regulating Akt activity, leading to cell proliferation and improving survival rates [41]. In the present study, we showed that overexpression of Trx-1 in Trx-1 Tg mice and pretreatment with rhTrx-1 in RGM-1 cells suppressed the indomethacin-induced decrease of survivin in gastric mucosal tissues and RGM-1 cells (Figure 6(A),(B)). It is the first demonstration that Trx-1 has an effect on the expression of survivin. These results indicate that Trx-1 may exert its protective function against indomethacin-induced gastric mucosal injury by

regulating the PTEN/Akt/survivin pathway, in addition to suppressing indomethacin-induced ROS generation.

We have shown that Trx-1 prevents indomethacin-induced gastric mucosal injury and the cytotoxicity by scavenging ROS and regulating the PTEN/Akt/surviving signal pathway. Geranylgeranylacetone (GGA), an acyclic polyisoprenoid, is widely used as an anti-ulcer drug. We previously reported that GGA can induce Trx-1 and protect gastric mucosal cells from ethanol-induced cytotoxicity [23,24]. Trx-1 and Trx-1 inducer such as GGA might have a potential for the prevention and treatment of gastric mucosal injury caused by NSAIDS and other stresses.

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