Suppressive effect of administration of recombinant human thioredoxin on cutaneous inflammation caused by UV

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Thioredoxin (TRX) is a small ubiquitous protein, which regulates cellular redox status and scavenges reactive oxygen species (ROS). TRX has been shown to exert suppressive effect on skin inflammation where oxidative stress is involved in its pathogenesis. We investigated the effect of TRX on UVB response. Ear swelling after UVB irradiation was significantly reduced in TRX-transgenic mouse compared with wild-type mouse. Furthermore, we have demonstrated that intraperitoneal administration of recombinant human thioredoxin (rhTRX) also reduced acute skin inflammatory reaction, such as skin erythema and edema. Histologically, inflammatory cells including neutrophils and lymphocytes were significantly reduced and average size of the caliber of blood vessels were also reduced in rhTRX-injected mice. The number of apoptotic keratinocytes, were significantly reduced in rhTRX-injected mice. Immunohistochemical intensity of 8-hydroxy-2'-deoxyguanosine was strikingly reduced in rhTRX-injected mouse. Western blotting showed that administration of rhTRX inhibited phosphorylation of p38 mitogen-activated protein kinases and c-Jun NH2-terminal kinase, which play important roles in inflammatory and apoptotic signaling. These findings indicated that rhTRX attenuated inflammatory and apoptotic responses by UVB. Possible mechanisms for this might be via redox regulation of stress signaling and reduction of reactive oxygen species. We discussed the future use of TRX for sedative use of skin inflammation.

Keywords: UVB, thioredoxin, oxidative stress, cytokine, neutrophil, JNK, Bcl-2, 8-OHdG, p38 MAPK, redox biology

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ADDENDUM

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induced tissue injury. In contrast, con-
tact hypersensitivity (CHS) reaction is a model for allergic CD, a cell-mediated immune type IV hypersensitivity, which is induced by the application of haptens on the skin. Exposure to antigenic stimulation by hapten stimulates cutaneous den-
ritic cells (DCs), which results in their migration from the skin into draining lymph nodes. Penetrating antigenic peptides to naive T cells. Once mice are sensitized, application of the hapten induces a local inflammatory response where memory T cells play a major role. CHS is not medi-
ated only by T cells but also by neutro-
phil infiltration to the hapten-challenge sites during the elicitation phase of CHS. Because patients with allergic CD have already been sensitized to antigens, avoidance of causal allergens is most important to prevent from the disease. However, this is not feasible in many cases because of occupational reasons and especially in cases where the contact allergen has not yet been identified. Thus, the develop-
ment of new therapeutic strategies in the elicitation phase of allergic CD is essential for clinical dermatologists.

Oxidative stress plays a key role in the inflammation of CD.13,14 We demonstrated that TRX suppresses the CHS response caused by DNPB and that the suppressive effect of TRX is mediated in the elicita-
tion phase but not the sensitization phase of CHS.14 It means TRX could suppress irritant dermatitis induced by irritant allergens, such as singlet oxygen, superoxide anion and hydrogen peroxide, subsequently resulting in alteration of their functions and resulting in activation of oxidative-stress-mediated transduction toward cell death and inflammation. It is recognized that a low level of oxidants can modify cell signaling via redox regulation and that these signal modifications have functional consequences.15 Mitogen-activated protein kinase (MAPK) pathway is the most sensi-
tive among MAPKs that are activated by proinflammatory cytokines and environ-
mental stress through phosphorylation. It has been reported that mice treated with p38 mitogen-activated protein kinase (p38 MAPK) inhibitor SB242235 showed marked inhibition of acute sunburn inflammation and apoptosis.17,18 Little study has been known about the effect of TRX on UVB-mediated skin injury although recent study showed that ROS mediated by UVB play an important role in acute inflammatory response caused by UVB. Therefore we evaluated the effect of TRX on acute skin inflam-
mation caused by UVB.16 We irradiated TRX-Tg mice and wild type mice with a single dose of 5 kJ/m2 of UVB and ear thickness was measured before and after UVB irradiation. Ear swelling increased until 120 h after UVB irra-
diation in a time dependent manner in both TRX-Tg and WT mice. However, the increment of ear thickness was much less in TRX-Tg mice compared with WT mice.

Since exogenously administered human TRX have been shown to suppresses lipopolysaccharide-induced neutrophil recruitment,19 we further investigated exogenously administered TRX had suppressive effect on acute UV response in murine skin. We used albino hairless mice for further investigation of the sup-
pressive effect of TRX on UV-induced inflammation to reproduce the human sunburn reaction because it is very dif-
ficult to see UV induced erythema using black hairy mice. Hairless albino mice were irradiated with eight graded doses of UVB on the dorsal skin. UV-induced erythema and edema was assessed 24 h after UVB exposure. The minimum ery-
thesia dose, slight but clearly discernible erythema were obtained by exposure to 750 J/m2. The dose of 750 J/m2 was used for further investigation to analyze the effect of TRX on UV response. Wild type mice were injected with rhTRX prior to 750 J/m2 of UVB exposure. PBS was served as control. Erythema was clearly attenuated by the administration of rhTRX at every time point observed. Dilatation of the vessel of the ear skin was visibly prominent in the PBS-injected mice whereas it was much less in rhTRX-injected mice. Ear swelling of rhTRX-injected mice was significantly suppressed compared with that of PBS-
injected mice at all time points (* p < 0.01, ** p < 0.05). For the histological evaluation, we obtained skin specimens from rhTRX-
induced and PBS-injected mice 24 h after irradiation with a single dose of 750 J/m2 of UVB. Skin specimens from the PBS-
 injected mice showed changes associated with skin inflammatory reactions, such as hyperkeratosis, acanthosis and exten-
sive cell infiltration in the epidermis and dermis. On the other hand such changes were greatly suppressed in rhTRX-injected mice. UVB induced neutrophil infiltra-
tion in the epidermis resulted in the for-
mation of epidermal microabscesses and such microabscesses were observed in the PBS-injected mice, whereas they could be hardly seen in rhTRX-injected mice. The number of neutrophils and lymphocytes were statistically significantly decreased in rhTRX-injected mice in comparison with PBS-injected mice.

In order to gain insights how rhTRX exerts its suppressive effect on the acute response mediated by UVB, we investi-
gated whether rhTRX affect the activa-
tion (phosphorylation) of p38 MAPK and JNK pathway. Western blot showed that p38 MAPK remarkably phosphorylated as early as 0.5 h after UV exposure and the phosphorylation was kept 3-fold increase above the baseline level at 2 h after UV exposure in both TRX-injected and PBS-
injected mice. However p38 MAPK phos-
phorylation was transient and returned to the baseline level within 24 h after UV exposure.
exposure in rhTRX injected mice, whereas high phosphorylation level persisted in PBS-injected mice. JNK phosphorylation was also significantly downregulated in rhTRX-injected group at 0.5 and 2 h post-irradiation compared with PBS-injected group.

In summary UV induced inflammation was significantly suppressed in TRX-Tg mice. Moreover, we demonstrated that systemic administration of rhTRX inhibited UV-induced inflammatory skin reaction in terms of erythema and swelling development. Histological analysis also confirmed that TRX attenuated the UV induced inflammation. Dermal infiltrating cells, both neutrophils and lymphocytes were significantly decreased in rhTRX-injected mice in comparison with PBS-injected mice. Histological analysis of human skin after moderate dose of UVB irradiation indicated that infiltration of neutrophil is a key molecule for the initiation of acute skin reaction mediated by UVB.20 Our data clearly showed that administration of rhTRX suppressed infiltration of neutrophils and lymphocytes at the UVB irradiated site 24 h after UV exposure. Our observations are consistent with previous findings that intravenous administration of rhTRX suppressed lipopolysaccharide (LPS)-induced neutrophilic infiltration into subcutaneous air-pouch in BALB/c mouse skin21 and that TRX suppresses the contact hypersensitivity response by inhibiting neutrophil recruitment during elicitation phase.22

Although the inflammation produced by UV has been well documented clinically and histologically, the mechanisms by which mediators induce response remain poorly clarified. Upon UV absorption by cellular molecule, photochemical reaction occurs, and this process should be responsible for initiating sunburn reaction. Devary et al. suggested that the UV response is initiated at or near the plasma membrane and the response may be elicited by oxidative stress.23 Indeed there are plenty of evidences that antioxidants attenuate erythema or edema mediated by UVB.24,25 We also confirmed that the administration of rhTRX remarkably decreased the formation of UV induced 8-OHdG, sensitive marker for oxidative stress, in murine epidermis whereas it had no effect on CPD and (6-4) photoproduct, majority of which are formed without ROS intervention.26 Our observations demonstrated that injected rhTRX showed marked anti-oxidant activity to scavenging ROS directly.

We further investigated the mechanisms how injected TRX attenuated UV-induced inflammatory and apoptotic response. UV acute response is elicited by oxidative stress, followed by activation of MAPKs, resulting in the activation of AP-1 transcription. MAPK are recognized as stress-activated proteins, being activated in response to various stimuli. Among them, p38 MAPK and JNK are the most sensitive of the MAPKs that are activated by proinflammatory cytokines and environmental stress through phosphorylation. In SKH mice, treatment with p38 MAPK inhibitor, SB242235, after UV irradiation lead decrease in the production of interleukin-6 and interleukin-8 and attenuation of UVB erythema. In our study, increase in p38 MAPK phosphorylation at 0.5 and 2 h after post-UVB irradiation were similar level between the two groups, followed by significantly lower level at 12 and 24 h post-irradiation in TRX-injected mice compared with that of control mice. These results including ours and others suggest that TRX attenuated UV-induced inflammation through downregulation of p38 MAPK pathway. It is known that reduced-form of TRX bind to apoptosis signaling-regulating kinase-1 (ASK1), a member of MAPKKK regulating p38 MAPK and JNK cascade, to inhibit its kinase activity. How extracellular TRX administrated may regulate intracellular redox signaling involving ASK system is to be clarified. We speculate that rhTRX ameliorated UVB-induced inflammatory response and apoptosis on murine skin, possibly through redox regulation of intracellular stress signaling and scavenging of ROS. Figure 1 summarizes the possible mechanisms of TRX on suppressive effect on acute UV response to the skin.

Taken together our recent findings together with others implies the possible usefulness of exogenously administered rhTRX for sedative effect on various skin inflammatory disorders induced by oxidative stress. However, long-term use of thioredoxin should be cautious for its possible promotion of carcinogenesis because of its anti-apoptotic effect. On the other hand, there is the opposite possibility. Accumulation of oxidative DNA damage plays an important role in UVB-induced skin cancer development.27 There are several evidences that sustained inflammation also promotes carcinogenesis.

Figure 1. Possible mechanisms of TRX on suppressive effect on acute UV response.
TNFα deficient mice are resistant to skin carcinogenesis. Overexpression of cyclooxygenase-2, one of the downstream proteins of p38 MAPK, enhanced UV-induced tumor development. In this context, it could be possible to suppress carcinogenesis promoted by oxidative damages and inflammation. Safety and effectiveness of long-term use of rhTRX for chronic sunburn exposure should be clarified for future study.

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