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Electron Microscopical Evidence of the Protective Function of Thioredoxin (TRX/ADF) Transgene against 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced Cellular Toxicity in the Liver and Brain

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Abstract: The present study was performed to assess the protective role of thioredoxin/adult T-cell leukemia-derived factor (TRX/ADF) on the liver and brain cell damages induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in ADF wild-type (WT) and transgenic (Tg) mice. The ADF WT and Tg mice were intraperitoneally injected with a single dose of TCDD (150 µg/kg body weight). One day after the treatment, the liver and brain tissues were examined electron microscopically to evaluate the cellular toxicity. In the ADF WT mice, marked reduction of subcellular components, such as mitochondria, rough endoplasmic reticula, and glycogen granules, as well as swelling of the remaining mitochondria, were evident in the liver cells. However, attenuation of these changes was evident in TCDD-treated TRX/ADF mice. Similar subcellular changes noted in the neuronal cells of TCDD-treated WT mice were also attenuated in Tg mice. The results suggest that oxidative cellular damage contributes to the acute toxicity induced by TCDD and that TRX/ADF protects against it. (J Toxicol Pathol 2005; 18: 41–46)

Key words: Ah receptor, brain, liver, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), thioredoxin/adult T-cell leukemia-derived factor (TRX/ADF), transgenic (Tg) mouse

Introduction

As one of the aromatic hydrocarbons, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a widely spread environmental pollutant that has a broad spectrum of toxic effects on a variety of tissues such as the thymus, liver, testes and central nervous system in mammals1–6. Although a number of studies have shown that the toxic effects of TCDD are mediated by intracytoplasmic aromatic hydrocarbon receptor (AhR)7–9, the toxic mechanism of TCDD on the target organs is still not fully understood. Among the toxic events, oxidative stress is considered to play a major role in the toxic mechanism of TCDD, as characterized by marked increases of lipid peroxidation, the formation of reactive oxygen species, and DNA single-strand break9–14.

Exogenous xenobiotics, such as aromatic hydrocarbons, result in profound induction of cytochrome P450 enzymes in the liver, resulting in the generation of reactive oxygen species15,16. On the other hand, the brain is rich in peroxidizable fatty acids and has relatively low catalase activity17. Therefore, these organs are considered to be highly susceptible to oxidative stresses18. In fact, the contribution of oxidative stress in TCDD-induced cellular damage of the liver and brain has been suggested in previous studies13,18–22.

Adult T-cell leukemia-derived factor (ADF) is a human thioredoxin (TRX) associated with the reduction/oxidation (redox) regulation of the cellular environment23. TRX/ADF is a stress-inducible protein and its expression is up-regulated after viral infection as well as in cellular stress conditions induced by oxidative agents such as hydrogen peroxide or diamide, irradiation with X-rays and ultraviolet
light, or ischemic reperfusion\textsuperscript{23}. Previous studies have shown that TRX/ADF plays a role in the cellular defense mechanism against oxidative cellular damage via the regulation of intracellular redox status, since exogenously administered TRX/ADF protected cells from oxidative cellular injury\textsuperscript{24,25}.

We recently reported for the first time the protective function of TRX/ADF against TCDD-induced hematotoxicity in ADF transgenic (Tg) mice, indicating oxidative stress contributes to the hematotoxic mechanism of TCDD\textsuperscript{26}. We hypothesized in the present study that overexpression of TRX/ADF might also be effective for protection against the toxic effects of TCDD on the liver and brain tissues in which oxidative stress has also been implicated in the toxic mechanism. For this purpose, we injected TCDD with a dosage capable of inducing oxidative stress in the liver following acute exposure\textsuperscript{21}, to ADF wild-type (WT) and transgenic (Tg) mice, and then compared subcellular changes electron microscopically in the liver and brain tissues.

Materials and Methods

Animals

TRX/ADF overexpressed mice (ADF Tg mice), originally produced by Dr. A. Mitsui\textsuperscript{27}, were maintained in a laboratory facility with a 12:12-hour light-dark cycle at an ambient temperature of 21 ± 2°C at the National Institute of Health Sciences (NIHS) of Japan by breeding ADF WT and Tg mice. Animals were screened by PCR of their tail DNA to determine their genotypes. At 8 weeks of age, male ADF WT and Tg mice (23.5–24.8 g) were transferred to a vinyl isolator established in a hazard room designed to prevent contamination from the outside environment and randomly allocated within the same genotype to housing with 6 animals per cage. A pelleted basal diet (CRF-1; Funabashi Health Sciences (NIHS) of Japan by breeding ADF WT and Tg mice. Animals were screened by PCR of their tail DNA to determine their genotypes. At 8 weeks of age, male ADF WT and Tg mice (23.5–24.8 g) were transferred to a vinyl isolator established in a hazard room designed to prevent contamination from the outside environment and randomly allocated within the same genotype to housing with 6 animals per cage. A pelleted basal diet (CRF-1; Funabashi Farm, Funabashi, Japan) and tap water were provided \textit{ad libitum} throughout the study.

Chemical

TCDD was obtained from Radian International, Cambridge Isotope Laboratories, Inc. (Andover, MA, USA; purity: 98 %). TCDD was initially dissolved in a small volume of acetone and subsequently adjusted to the concentration of 10 µg/ml in olive oil.

Experimental design

ADF WT and Tg mice were divided into vehicle controls and TCDD treatment groups, each consisting of 6 animals. After one week of acclimation, TCDD at 150 µg/kg was intraperitoneally injected once to animals of treatment groups, and the corresponding volume of olive oil was similarly injected to vehicle controls. The dosage of TCDD was selected based on previous study results that showed oxidative stress in the liver was induced by a single bolus injection to mice\textsuperscript{21}. One day after the treatment, the animals were sacrificed by decapitation and then examined grossly.

The liver and brain were then excised and their weights were measured.

The animal protocol was reviewed and approved by the Animal Care and Use Committee of the NIH, Japan.

Morphological assessment

For histological examination, liver tissues in all animals were fixed in 10% neutral buffered formalin (pH 7.4). After routine processing, the paraffin-embedded sections were stained with hematoxylin and eosin and then examined histopathologically under a light microscope.

For electron microscopical examination, tissue specimens from the liver and cerebral cortex were respectively prepared from three animals each of the control and treatment groups of ADF WT and Tg mice. Small tissue blocks, sized 1 mm\textsuperscript{3}, were fixed with 2.5% glutaraldehyde in 0.2 M Sorenson’s sodium phosphate buffer, pH 7.2, for 8 hours at 4°C. After washing with 0.1 M PBS (pH 7.4), the tissues were post-fixed with 1% osmium tetroxide for 90 minutes. After washing in 0.1 M PBS, the tissues were dehydrated with ethanol and propylene oxide and then embedded in Epon 812. Ultrathin sections were double-stained with uranyl acetate and lead citrate. The sections were examined with JEOL-1200 EX II electron microscope (JEOL, Tokyo, Japan).

Results

After one day of TCDD treatment, absolute liver weight had decreased to 71.4% of the vehicle control group in ADF WT mice and 83.2% in ADF Tg mice (data not shown).

Histologically, apoptotic liver cell debris and also focal liver cell necrosis were sparsely observed in the centrilobular areas of both TCDD-treated WT and ADF Tg mice, without showing apparent difference in the severity between genotypes (data not shown). Vehicle control animals did not show such liver cell changes in either genotype.

Electron microscopically, liver cells of the WT mice treated with TCDD exhibited a prominent decrease of cytoplasmic glycogen granules and rough endoplasmic reticula (RERs) and an increase of smooth endoplasmic reticula (SERs) (Fig. 1B). The number of mitochondria was also decreased and the remaining mitochondria showed swelling with disorganized cristae and lucent matrix. Increased fat droplets were also evident in the cytoplasm of less affected hepatocytes. On the other hand, transgene of Trx/ADF notably attenuated these morphological changes following TCDD treatment (Fig. 1C). In the cerebral cortex, neuronal cells showed a decrease in the number of RERs, ribosomes and mitochondria in WT mice treated with TCDD (Fig. 2B) but not in ADF Tg mice treated similarly with TCDD (Fig. 2C). Vehicle control animals did not show such neuronal cell changes in either genotype.

Discussion

In the present study, acute treatment with TCDD
Fig. 1. Electron micrographs of liver cells from ADF WT and Tg mice treated with vehicle or TCDD. (A) Vehicle-treated ADF WT mouse, (B) TCDD-treated ADF WT mouse, and (C) TCDD-treated ADF Tg mouse. Note cytoplasmic swelling associated with a profound decrease of glycogen granules, RERs and mitochondria in the liver cells of the TCDD-treated ADF WT mouse (B). Swelling of the remaining mitochondria with disorganized cristae and lucent matrix is also evident (B). Attenuation of these morphological changes is evident in the TCDD-treated ADF Tg mouse (C). Uranyl acetate and lead citrate. Bar=10 µm (A1, B1, C1), Bar=3 µm (A2, B2, C2).
Fig. 2. Electron micrographs of neuronal cells in the cerebral cortex from ADF WT and Tg mice treated with vehicle or TCDD. (A) Vehicle-treated ADF WT mouse, (B) TCDD-treated ADF WT mouse, and (C) TCDD-treated ADF Tg mouse. Note the decrease of RER, ribosome and mitochondria in the cytoplasm of neuronal cells of the TCDD-treated ADF WT mouse (B). In the TCDD-treated ADF Tg mouse, mitochondrial swelling is also evident, but attenuation of the morphological changes can be seen, too. (C). Uranyl acetate and lead citrate. Bar=10 µm (A1, B1, C1), Bar=2 µm (A2, B2, C2).
induced ultrastructural alterations in the cytoplasmic components of liver cells characterized by prominent decrease of glycogen granules and RERs, proliferation of SERs, decrease and degradation of mitochondria, and increase of lipid droplets. These subcellular alterations were mostly consistent with those noted in the guinea pig liver following TCDD treatment, but concentric membrane arrays in the liver cells were not evident in the present study, presumably due to the different experimental protocol or the different species used in the studies. In the cerebral neuronal cells in the present study, alterations in subcellular components by TCDD were also evident, despite the changes being less profound than those in the liver cells. These subcellular changes in the liver and neuronal cells may represent the cytotoxic outcome of TCDD due to oxidative cellular damage and also cellular adaptation including detoxification.

Effective prevention of TCDD-induced toxicity by administration of antioxidants such as oltipraz-[5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione] or butylated hydroxyanisole, or by pretreatment with vitamins A and E further supports the hypothesis that oxidative processes are involved in TCDD-induced toxicity. Attenuation of subcellular changes in the liver and neuronal cells by transgene of TRX/ADF in the present study indicates the critical role of oxidative stress in the toxic events induced by TCDD, and also the protective function of ADF/TRX in these organs, as in our previous study of TCDD-induced bone marrow toxicity. The protective effect of TRX/ADF against oxidative cellular damage is believed to be achieved by free radical scavengers, activation of DNA repair enzymes, such as activator protein endonuclease (Ref-1; redox factor-1) and activation of nuclear factor-kappa B (NF-kB). Taken together, the results of our present study strongly suggest that the acute toxic effect induced in the liver and brain by a single large dose of TCDD is due to oxidative cellular damage, and that TRX/ADF plays a role in protection against TCDD-induced acute toxicity. Considering the routes and concentrations of TCDD exposed to humans, research on the effect of extremely low doses of TCDD by oral ingestion on the oxidative cellular damage of target organs is clearly warranted.

References