TRANSGENE EXPRESSION OF THIOREDOXIN (TRX/ADF) PROTECTS AGAINST 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD)-INDUCED HEMATOTOXICITY

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Introduction

TCDD has a broad-spectrum of toxic effects on a variety of organs including hemopoietic system[1,2]. TCDD exposure induces a decrease in bone-marrow (BM) cellularity and CFU-GM[2]. Oxidative stress has been implicated in the mechanism of TCDD toxicity in liver and brain[3,4]. BM is exquisitely sensitive to oxidative stress, because leukocytes are a primary source of oxygen radicals. However, it has still not been studied if oxidative stress would play an important role in TCDD-induced hemopoietic toxicity.

Adult T-cell leukemia-derived factor (ADF), an inducer of interleukin-2 receptor, is a thioredoxin (TRX) associated with the reduction/oxidation (redox) regulation of the cellular environment[5]. TRX/ADF is a stress-inducible protein whose expression is up-regulated by viral infection as well as by cellular stress induced by a variety of oxidative agents[5]. TRX/ADF has been shown to be involved in the cellular defense mechanism against oxidative damage via the regulation of intracellular redox status[5].

The present study was performed to evaluate the role of oxidative stress and the protective effect of TRX/ADF on TCDD-induced hematotoxicities using ADF wild-type (WT) mice and ADF transgenic (Tg) mice.

Materials and methods

Eight-week-old male WT and Tg mice (23.5-24.8 g) were maintained and treated in a vinyl isolator designed to prevent environmental exposure to TCDD for the duration of the study. After 1-week acclimation, they received a single intraperitoneal injection of either a 20μg/kg dose of TCDD (treatment group) or olive oil containing the acetone carrier (control groups). Three mice were used per group. Food and tap water were given *ad libitum* during the study. TCDD was dissolved in a small volume of acetone and subsequently adjusted to a working concentration of 1 μg/ml in olive oil. One day after the treatment, peripheral blood was collected from the orbital plexus and then blood parameters were measured using a Sysmex M-2000 blood cell counter. BM cells were flushed out of femurs with 2 ml of cold Dulbecco-modified Minimum Essential Medium (GIBCO, USA) and nucleated cells were counted using a Sysmex M-2000 counter. For *in vitro* colony assay for CFU-GMs, semisolid medium containing 0.8% methylcellulose, 30% fetal calf

serum, 1% bovine serum albumin, 10^{-4} M 2-mercaptoethanol, and 1 ng/ml interleukin-3 (IL-3) and 2 u/ml erythropoietin (EPO) was used. The culture medium added with 2×10^{4} cells/plate was incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Colonies were enumerated under an inverted microscope on day 6 after plating.

Western blot electrophoresis was carried out to analyze the expression of the human TRX (hTRX) in the BM cells of WT and Tg mice. Protein samples of 5 μg were subjected to 12%(w/v) SDS-polyacrylamide gel electrophoresis, and then transferred to Hybond™ PVDF membranes. After blocking nonspecific binding-sites with 5 % non-fat dried milk, the membranes were incubated with the murine anti-hTRX, followed by incubation with horseradish peroxidase-conjugated secondary antibody. The membranes were visualized with a detection reagent and then the densities of bands were measured using an image analyzer.

To evaluate the level of AhR mRNA expression in the BM, 5-μg total RNA that was extracted from BM cells was reverse-transcribed and then amplification by PCR was performed using oligonucleotide primers specific for mouse Ah receptor (5`primer (5`-GAT GCC TTG GTC TTC TAT-3`) and 3` primer (5`-TCA TGC CAC TTT CTC CAG TCT-3`)). A 10-μl of PCR products was electrophoresed in 1.5 % agarose gel in Tris-acetate/EDTA electrophoresis buffer.

Results and discussion

The decrease of BM cellularity and suppression of colony formation of hemopoietic progenitor cells (CFU-GM and CFU-E) were previously demonstrated in TCDD-responsive mice after short term TCDD exposure[2]. However, studies regarding the role of oxidative stress in the hematotoxic mechanism of TCDD have not been reported previously in the literature. In this study, to explore the role of oxidative stress and the protective function of TRX in the hematotoxic mechanism of TCDD, WT and hTRX Tg mice (Figure 1) were treated with TCDD at a dose of 20 μg TCDD/kg, which induced severe decrease in BM cellularity and CFU-GM (to 73.4±5.5% and 64.4±9.8% of control values, respectively) and in the number of peripheral leukocyte (to 71.3±4.2% of control value) in the WT mice (Figure 2A). However, no comparable toxic effects of TCDD on blood and BM were observed in the Tg mice (Figure 2A). These findings strongly suggest that oxidative stress plays an important role in the TCDD-triggered mechanism of hematotoxicity, an effect comparable to that reported by others to occur in liver and brain[3,4]. Furthermore, our results suggest that disruption of redox regulation may be induced by TCDD. Macrophages and neutrophils have a unique O_2^- producing system, known as respiratory bursts, that has been reported previously to be activated by TCDD treatment[6]. The protective effect of TRX/ADF against oxidative stress-induced cell damage has been shown to be achieved by a free radical scavenger and/or by activation of a DNA-repair enzyme such as activator protein (AP) endonuclease (redox factor-1: Ref-1)[5]. TRX/ADF may also have its protective function against TCDD-induced hematotoxicity via regulation of the activation of nuclear factor-kappa B (NF-kB) that is induced by reactive oxygen intermediates[5]. It has recently been reported that NF-kB can be activated by TCDD treatment[7], and TRX had been shown to suppress the activation of NF-kB[8].

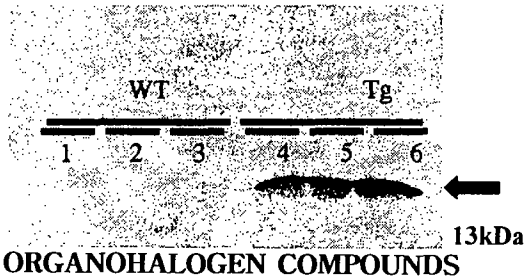

Fig. 1. The expression of hTRX in BM cells of WT and Tg mice. In Tg mice, signal of hTRX transgene was strongly expressed in BM cells but undetectable in WT mice. Western blotting analysis (protein load: 5 μg).

There was no difference in the expression of AhR mRNA in BM cells between untreated WT and Tg mice (Figure 2B). It is not surprising that there was no change of AhR mRNA level following TCDD treatment in the BM cells of WT mice in this study, since the regulation in AhR mRNA level following TCDD treatment has been shown to be tissue specific. Not only increase (in liver) or down-regulation (in craniofacial tissue) but also no change (in reproductive organs) in the mRNA level of AhR had been reported in the target organs of rats and mice following TCDD treatment[9,10]. On the other hand, it was interesting that the level of AhR mRNA was down-regulated in the BM cells of Tg mice which were prevented from TCDD-induced BM toxicity (Figure 2B), suggesting a possible interaction between AhR and TRX/ADF in the protective mechanism of TRX/ADF even if further studies are required to define this relationship in more detail.

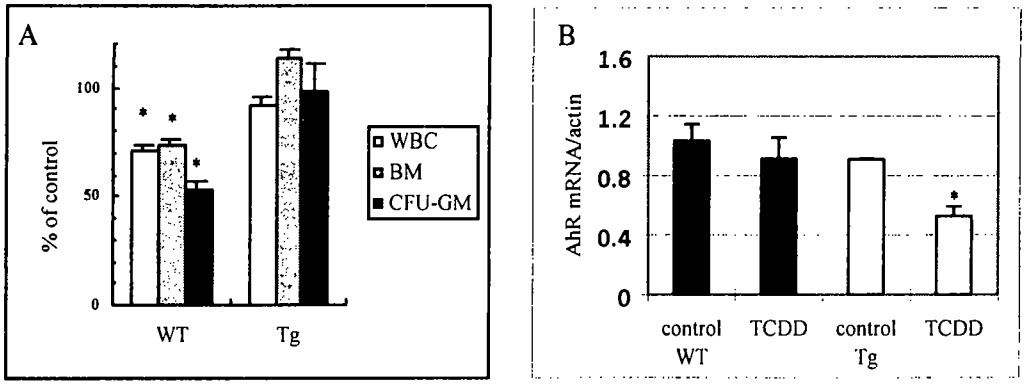

Fig. 2. Changes of circulating leukocyte (WBC), bone-marrow cellularity (BM) and CFU-GM/6×10^4 BM cells (CFU-GM) in TCDD-treated WT and Tg mice (A) and down-regulation of AhR mRNA in TCDD-treated Tg mice (B). Femoral BM cells were respectively harvested from WT and Tg mice one day after a single IP injection of 20 μg/kg of TCDD. Values represent mean±SEM of three mice per group. * $p<0.05$ with respect to control group in student T-test.

TCDD-induced oxidative stress is believed to be mediated by AhR. Alsharif *et al.* (1994) reported that the dose of TCDD required to induce a significant increase of superoxide anion formation is 25 fold higher in TCDD-non-responsive mice than in responsive mice[11]. Therefore, TRX/ADF may exert its protective effect in the AhR-mediated pathway through which TCDD induces oxidative stress.

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