The oscillating recovery in cellularity of the bone marrow and CFU-GMs after intraperitoneal 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure

Byung-Il Yoon, Yoko Hirabayashi, Yukio Ogawa, Jun Kanno, Tohru Inoue, Toyozo Kaneko

Cellular and molecular toxicology division, National Institute of Health Sciences, Tokyo, Japan

Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a wide spread environmental pollutant and has toxic effects on various organs including the liver, thymus, testes and the central nervous system. Hemopoietic system has also been regarded as one of the targets of TCDD in laboratory animals and monkeys (1,2). Acute or subchronic administration of dioxin induces a decrease in cellularity of the bone marrow, red blood cells and thrombocytes (2,3,4). However, concerning the number of leukocytes in the peripheral blood, the subchronic or chronic administration of low dosages of TCDD did not show any significant change (3,4) but rather increased (5), although a high dosage of TCDD could induce severe myelosuppression (2). Therefore, the hemopoietic cell kinetics including marrow celluarity and the response of CFU-GM may be important to understand and to evaluate the risk of the mechanistic background of the damage of the hemopoietic system. According to the cell kinetics reported by Luster *et al*., the cellularity of bone marrow and CFU-GM was markedly depressed after the acute administration of high dosage of TCDD but tended to recover rapidly. However, the complete recovery from the depression was not observed in their report. The recovery pattern from the induction of toxicity may be useful to understand the toxic mechanism and to assess the risk of chemicals. Present study is the cell kinetics from the severe depression in cellularity of bone marrow and CFU-GM to their recovery after the intraperitoneal injection of high dosage of TCDD. Our cell kinetics certified the oscillation in bone-marrow celluarity and CFU-GM during the recovery period, of which the observation seems to be useful to extend our understanding in the hematotoxicity of TCDD.

Materials and methods

Eight-week-old male C57BL/6 mice (23.5-24.8 g) were used in this study. Animals were maintained and treated in a vinyl isolator established in the hazard room designed to prevent an environmental exposure. TCDD was initially dissolved in a small volume of acetone and subsequently adjusted to a working concentration, 1μg/ml, in olive oil. TCDD, 10μg/kg body weight (10^5 times of TDI in humans), was singly injected intraperitoneally on 12 day, 8 day, 4 day, 2 day, 1 day and 6 hours prior to killing mice and collecting bone-marrow cells. For control mice, olive oil containing the same concentration of acetone as the treated group was injected according to the same protocol. Bone-marrow cells were collected from two femora and counted using a Coulter Counter. For *in vitro* colony assay for CFU-GM, the semisolid medium containing 0.8 % methylcellulose, 30 % fetal calf serum, 1 % bovine serum albumin, 10^{-4} M 2-mercaptoethanol, and 1 ng/ml IL-3 was used. The culture medium added with 8×10^4 cells was incubated at 37 ^{o}C with 5 % CO_2 in the humidified atmosphere. The number of colonies developed was counted under inverted microscope on day 6 after plating.

Results and discussion

As shown in Figure 1, cellularity of the bone marrow and CFU-GM per femur was decreased to 60.1 % and 72. 3 % of the control value, 6 hours after injection of TCDD, respectively. The decrease of bone-marrow cellularity and CFU-GM was the most prominent on day 1 after exposure to TCDD, i.e., 37.8 % and 48 % of the control respectively. Afterwards, the depressed cellularity of the bone marrow was recovered rapidly and even overshot to reach about 110 % of the control value on day 4 after TCDD injection (see, Figure). In CFU-GM, the depression was also soon recovered and even overshot to reach about 151.9 % of the control value within 3 days after the bottom; subsequently returned to the control value. It was noteworthy that reovershoting of the bone marrow tended to decrease and oscillate again to the control value on day 8 and decreased down to 81.9 % by day 12 after TCDD injection. The severe decrease of bone-marrow cellularity and CFU-GM on day 1 after TCDD injection and its rapid recovery was similar to Luster *et al.*'s results although their application route was oral administration. The oscillation was more exaggerated in the present study possibly because of the different route of application, and

this oscillation was observed first in the literature as shown in Figure 1 in which cellularity of the bone marrow and CFU-GM was severely depressed first and overshot the control values, then was subsequently decreased again under the control during the recovery. It is of interest to observe a long-term effect of the recovery because the number and quality of the hemopoietic stem cells were not fully recovered after exposure of a single dose of radiation. In conclusion, present our study confirmed the kinetics of hemopoietic tissue including CFU-GM after TCDD exposure that may be useful to extend our understanding the hemoatotoxicity after TCDD exposure.

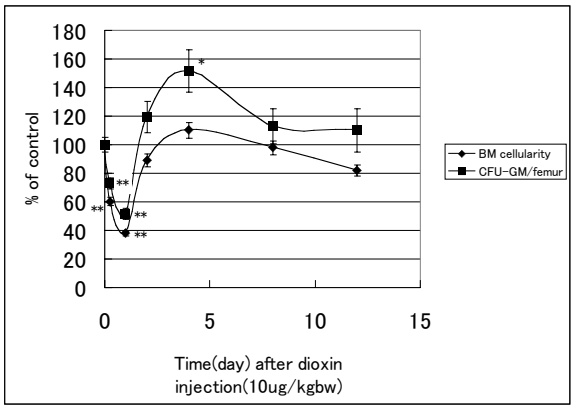

Figure 1. The panoramic changes in bone-marrow cellularity and CFU-GM / femur after TCDD exposure. TCDD, 10 μg/kg body weight, was singly and intraperitoneally injected to mice. Each point represents the mean±SEM from three mice. In control mice, the mean bone-marrow cellularity was 37.6×10^6 per femur and for CFU-GM 55 per 8×10^4 marrow cells.

* , ** ; significant different from control values at $p<0.05$ or $p<0.01$.

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