THYMIC ATROPHY IN RATS AFTER SINGLE ORAL INTUBATION OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was shown to be immunotoxic in all species investigated[1]. In rodents, atrophy of the thymus occurs after exposure to sublethal doses of TCDD. Within the thymus, the target cell population remains a matter of controversy[2]. TCDD has been reported to affect both the lymphoid cells and the epithelial stroma of the thymus. *In vitro* studies have provided evidence for the thymic epithelium as a target for TCDD-induced toxicity[3]. Higher TCDD concentrations were necessary to exert an effect on lymphoid cells[4]. Several *in vivo* studies point to the cortex area of the thymus as the site of action for TCDD. Acute exposure to TCDD in mice has been shown to decrease the percentage of immature $CD4^+CD8^+$ double-positive (DP) thymocytes, which reside in the thymus cortex[5]. In addition, in rats exposed to a single oral intubation of TCDD, a reduction in the relative cortex surface area, and alterations in cortical thymic epithelium were observed after immunohistochemical and electron microscopical examination of TCDD-exposed thymuses[6]. However, the *in vivo* studies mentioned above were performed using doses of several times the ED50 for thymic atrophy in rats and mice. Therefore, we extended these studies, by investigating thymic atrophy in rats exposed to a wide dose range of TCDD, starting at lower dose levels (1 μg TCDD/kg body weight).

Materials and methods

Chemicals. TCDD (>98% purity) was obtained from C.N. Schmidt B.V.,

Amsterdam, The Netherlands). The compound was dissolved in olive oil as vehicle (Brocacef, Maarssen, The Netherlands.

Experimental conditions. Male 4 weeks old SPF-derived Wistar rats (RIV:Tox) were obtained from the breeding colony of the National Institute of Public Health and Environmental Protection (Bilthoven, The Netherlands). The animals were kept in stainless-steel wire cages at an artificial 12-hr light/dark regimen (temperature, 22 ± 2°C; relative humidity, 45-65%). Because of the high toxicity of TCDD, all wire cages were placed in Gustafson isolators. Drinking water and food were provided *ad libitum*.

Animals were exposed to 0, 1, 5, 25, 50 or 150 µg TCDD/kg body weight (n=4) by gavage. The dosing volume was 10 ml/kg body weight. At autopsy on day 10, body weights were measured, and thymuses removed. After weighing, the thymus lobes were fixed in buffered formalin and embedded in paraplast; 3-µm sections were stained with hematoxylin and eosin for conventional light microscopy.

Relative cortical and medullary surface areas of thymic parenchyma were assessed on 3-µm paraffin sections for each rat thymus by morphometry, using an automatic image analyzer (IBAS 2000, Kontron, Munich, FRG). A minimum of three representative sections of each tissue specimen were measured.

Statistical analysis of the data was performed using analysis of variance ANOVA (oneway) with the Minitab statistical software package (Minitab Inc., State College, PA, USA). The level of significance was $p<0.05$.

Results

General toxicity. Significant differences in body weight were seen on day 10 after exposure between control animals, and animals treated with 50 or 150 µg TCDD/kg body weight. No differences were noted between the control group and groups exposed to 1-25 µg TCDD/kg body weight (data not shown).

Thymic weights. Absolute thymic weights showed a dose-dependent decrease (Fig.1). This reduction was significant after intubation of at least 25 µg TCDD/kg body weight. When thymic weights were expressed as relative thymic weights (% of body weight), significant reduction of thymus weights was already observed after a single oral intubation of 5 µg TCDD/kg body weight.

Histology. Thymuses of control animals showed normal histology of an uninvoluted thymus. In TCDD-exposed rats, a dose-dependent reduction in the size of individual thymic lobules, which parallelled the reduction in thymic weights, was observed. Significant differences in the relative cortical surface area of thymic parenchyma were noted only after intubation of 150 µg TCDD/kg (Fig.1). No effects were seen on the number of pycnotic nuclei scattered in the cortex, nor on the number of (starry-sky) macrophages containing phagocytozed dead cell remnants.

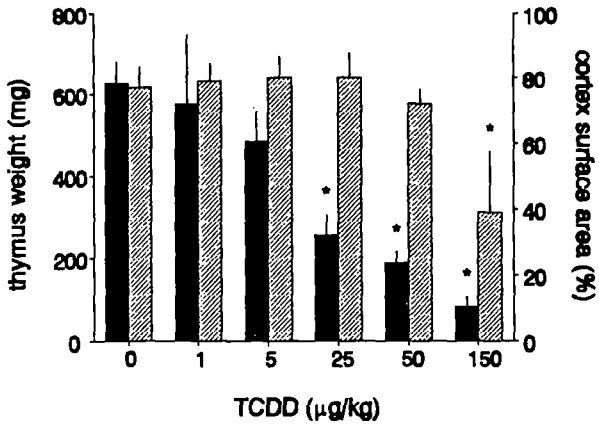

Figure 1.
Absolute thymic weights (closed bars) and relative cortex surface areas (hatched bars) in rats on day 10 after a single oral intubation of TCDD (means±SD). Asterisk values are significantly different ($p<0.05$) from the control values.

Discussion

The present data do not show an effect of TCDD on the thymus cortex to medulla ratio after exposure to lower dose levels of TCDD. Although a 70% reduction in thymic weight was observed after exposure to 50 µg TCDD/kg body weight, the relative cortex surface area of thymic parenchyma remained within control values. When higher doses (150 µg/kg) were used, TCDD-toxicity was more profound in the cortical area of the thymus.

The latter finding confirms previous results by our group[6], and suggest a relative decrease of immature cortical thymocytes, as was found in mice after exposure to higher doses of TCDD[5]. The observation that a preferential effect in the thymus cortex is not found after exposure to lower dose levels of TCDD may be explained by assuming that a preferential cortical lymphodepletion has indeed occurred, but that this effect is masked by an influx, and subsequent differentiation, of bone marrow-derived thymocyte precursors. This implicates that prothymocytes are not as sensitive to the action of TCDD compared to the thymus target cell population. Impairment of prothymocyte activity after TCDD exposure has been described in mice[7], but these effects were noted using relatively high doses of TCDD. The results of our previous studies[6] showed a tendency (not significant) for a lower relative cortex surface area on day 4 after a single oral intubation of 50 µg TCDD/kg body weight, compared to day 10 after administration, which would be in line with this explanation, i.e. by day 4 after administration recovery due to an influx of bone marrow precursors has not yet occurred. A second explanation for the absence of a decrease in cortex to medulla ratio may be that an initial cortical lymphodepletion has occurred, but is followed by an arrest in cellular traffic from cortex to medulla, thereby leading to a medullary lymphodepletion. In this way, effects secondary to the initial cortical lymphodepletion, may mask a preferential effect on the thymus cortex. An third possibility is, that the main target for the

action of TCDD does not reside within the thymus cortex. If this were true, thymocytes or epithelial cells should be equally sensitive to the action of TCDD throughout every differentiation stage of thymocyte development to explain the absence of either a preferential medullary or cortical lymphodepletion.

A time-response study after a single oral intubation of TCDD may help to discriminate between the options mentioned above.

Taken together, our data indicate that on day 10 after exposure to lower levels of TCDD, thymic atrophy is observed in the absence of a preferential decrease in the relative cortex surface area. Whether a preferential cortical lymphodepletion has occurred after a shorter period after dosing of lower doses of TCDD or not, remains subject for further studies.

Acknowledgement

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