Toxicological Characterization of 2,3,7,8-Tetrafluorodibenzo-p-dioxin (TFDD)

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1. Introduction

Among the polychlorinated and polybrominated dibenzo-p-dioxins, the 2,3,7,8-substituted congeners are the biologically most potent compounds. 2,3,7,8 -TCDD and related compounds act as tumor promoters in rodent liver[1,2], and exert a characteristic pattern of acute and subchronic toxicity [3]. Most effects of TCDD are thought to be mediated by binding/activation of the cytosolic dioxin receptor which acts as a nuclear transcription factor[4]. However, no information is available on the biological and toxicological effects of fluorinated dibenzo-p-dioxins. Therefore, we synthesized the fluorinated TCDD analogue 2,3,7,8-tetrafluorodibenzo-p-dioxin (TFDD) and examined its fate in rodents and in rodent hepatocytes in primary culture. In vivo in mouse blood and liver TFDD showed an elimination half-life of 5 - 7.5 hrs. As parameters for the biological potency of TFDD, effects on a minimal XRE (xenobiotic-responsive element)-driven promoter transfected into Hepa-1c-1c-7 cells, and induction of CYP1A-catalyzed 7-ethoxyresorufin O-deethylase (EROD) in rat hepatocytes in primary culture were examined. It was found that TFDD acts as a potent TCDD-like agonist in both systems.

2. Methods

TFDD was synthesized by pyrolysis of 2,4,5-trifluorophenol in the presence of KOH. The pyrolysate was extracted with toluene, and TFDD was isolated by liquid chromatography on Alumina B Super I (ICN Biomedicals, Wesel, Germany). TFDD was identified by high-resolution gas chromatography/low-resolution mass spectrometry and NMR.
Male NMRI mice (20-23 g body weight) were treated with a single i.p. dose of 100 μg TFDD/kg. At various time points, animals were anesthetized with ether and blood samples were taken. Then animals were sacrificed, and livers were removed for TFDD analysis. 72 h after treatment, livers were removed for fluorimetric determination of 7-ethoxyresorufin O-deethylase (EROD) in homogenates.
Hepatocytes were isolated from male Wistar rats (220-250 g body weight) as

described[5], and were plated at a density of 100,000 cells per cm^2 on collagen-coated petri dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal calf serum, 10 % calf serum, and 10^{-7} M dexamethasone. After 24 h in culture, various concentrations of TFDD, freshly dissolved in DMSO, were added, cells were harvested 48 h later, and EROD activity was determined in cell homogenates. For comparison, hepatocytes were incubated with TCDD. Log probit functions were fitted to the data by use of SAS probit procedure which also allows estimates for EC_{50} values.

Transcriptional activation of an XRE-driven promoter construct was studied in Hepa-1c-1c-7 cells transiently transfected with a wild-type or mutant[6] XRE reporter gene construct containing a minimal mouse mammary tumor virus (MMTV) promoter and a phosphatase reporter gene.

The fate of TFDD in culture medium was investigated by incubation of 12.5 μM TFDD in sterile serum-supplemented DMEM under humidified air:CO_2 atmosphere (5% CO_2) at 37°C.

The blood and liver samples were ground with Na_2SO_4, mixed with a definite amount of octafluorodibenzo-p-dioxin as internal standard, and extracted in a Soxhlet apparatus with toluene. After clean-up, the quantification was carried out using gas chromatography/mass spectrometry.

3. Results

After i.p. injection of male NMRI mice with 100 μg TFDD/kg dissolved in corn oil, apparent biphasic kinetics of elimination from blood and liver were obtained (Fig. 1).

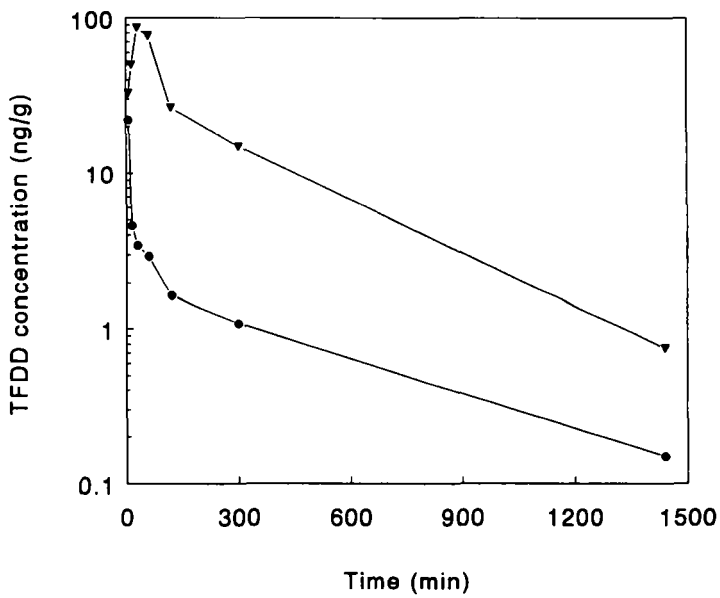

Fig. 1. Time course of TFDD level in blood (-●-) and liver (-▼-) of male NMRI mice after i.p. injection of a single dose of 100 μg/kg.

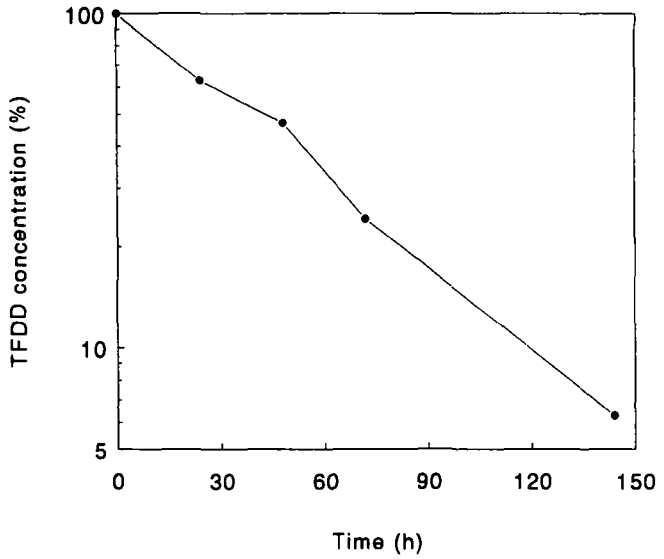

Fig. 2. Fate of TFDD in DMEM. The initial concentration was 12.5 μM.

From a semi-logarithmic plot of blood concentrations, elimination half-lifes of 5 min (rapid phase) and 7.5 h (slow phase) were calculated. In liver the TFDD level reached a maximum 30 min after injection, and also declined in a biphasic manner. For the slow phase of elimination from liver a half-life of ca. 5 h was estimated. No EROD induction was detectable in liver homogenates, 72 h after treatment. Likewise, no effects on EROD activity were seen in liver homogenates of male Wistar rats 72 h after treatment with 1 and 10 μg TFDD/kg.

In cell-free sterile DMEM in culture dishes, TFDD disappeared according to first-order kinetics (Fig. 2) showing a half-life of approximately 36 h. Preliminary studies in open and closed systems suggest that TFDD evaporates from the culture medium.

In Hepa-1c-1c-7 cells transiently transfected with a XRE-driven reporter gene, TFDD acted as a strong agonist showing a ca. 10-fold lower potency than TCDD (Fig. 3). Mutation of the dioxin receptor core sequence TCACGC to TCACTC totally abrogated transcriptional activation.

48 h after addition to rat hepatocyte cultures, EROD activity was significantly increased in a concentration-dependent manner (Fig. 4). At 10^{-9} M and at higher concentrations, the efficacy of induction markedly declined. Using a log-probit fitting procedure EC_{50} values were estimated for TFDD (1.0×10^{-11} M) and TCDD (1.9×10^{-11} M).

4. Discussion

Aromatic substitution of chlorine by fluorine usually leads to a slightly decreased lipophilicity, whereas an increase in volatility is frequently observed. Lateral

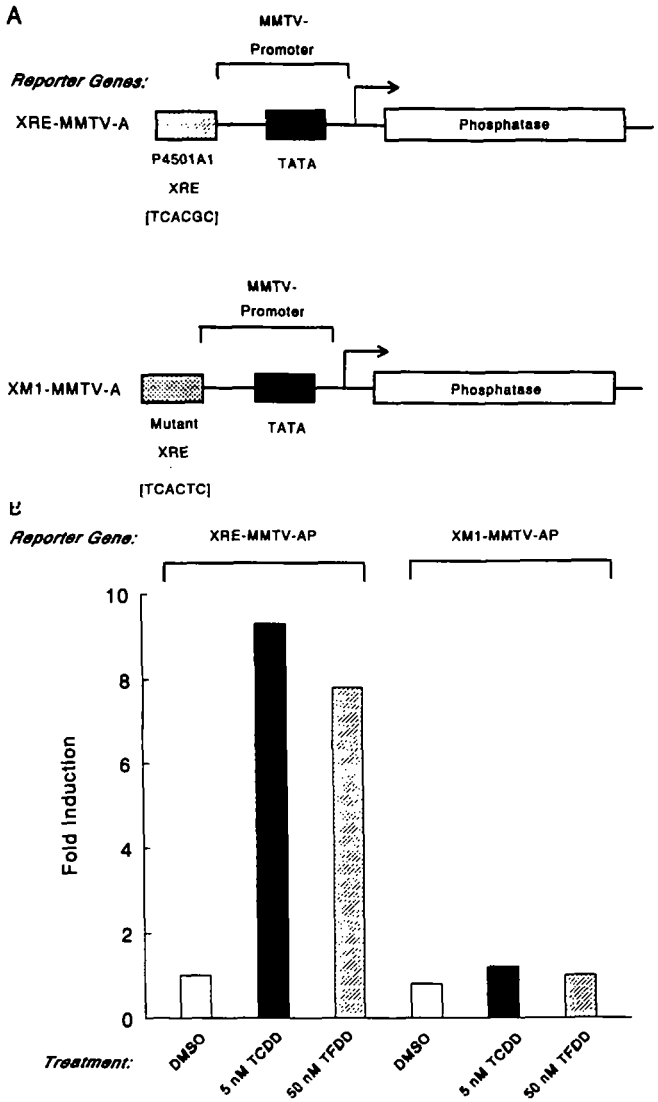

Fig. 3. Structure (A) and transcriptional activation (B) of a wild-type and mutant XRE-driven reporter gene transfected into Hepa-1c-1c-7 cells. Cells were incubated for 48 h.

fluorination of dibenzo-p-dioxin resulted in a dramatically reduced elimination half-life compared to the chlorinated analogue TCDD. In mouse blood (and liver) it was estimated to be ca. 5 h (7.5 h) compared to 8.5 days reported for TCDD[7] (in livers of male C57BL/6J). The rapid phase of TFDD disappearance from blood may be due to a transfer of the compound into the deep compartment, while the slow phase was

suggestive of a metabolic degradation or another long-term elimination process. Incubations of TFDD in cell-free medium revealed a non-enzymatic elimination, and experiments in open and closed systems suggest that TFDD evaporates from the medium. Likewise, TFDD may be exhaled in vivo, partially explaining its short half-life in mice, and the lack of CYP1A induction. Currently the possible role of metabolic degradation of TFDD is also under investigation.

In rat hepatocytes in primary culture, induction of CYP1A-catalyzed EROD activity could be demonstrated indicating that TFDD activates the dioxin receptor. This conclusion was confirmed by studies showing specifically enhanced transcription of a construct containing an XRE upstream of a heterologous promoter and a reporter gene.

Based on initial concentrations in rat hepatocyte cultures, similar EC_{50} values were found for TFDD and TCDD. These data would allow the calculation of a TCDD equivalency factor (TEF) of ca. 1.0. However, as a consequence of the short half-life of TFDD in mouse blood and liver, the rational basis for the calculation of a TE factor seems to be questionable and has to be extended by further investigations.

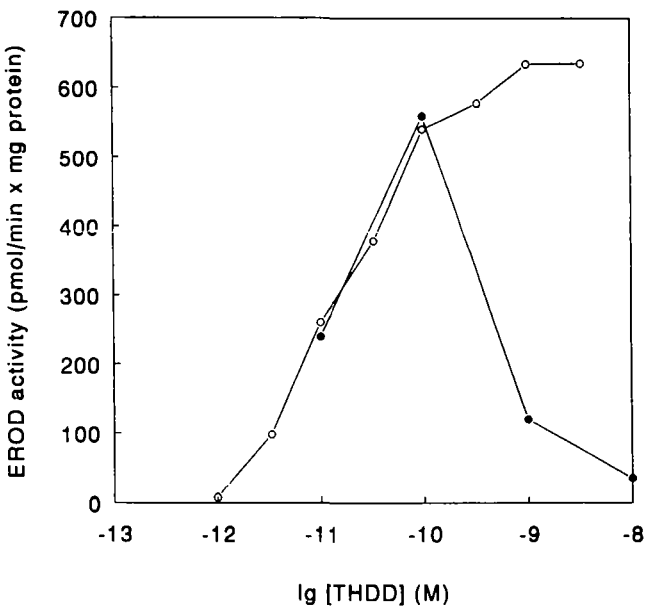

Fig. 4. Induction of 7-ethoxyresorufin O-deethylase (EROD) activity with TCDD (-o-) and TFDD (-●-) in rat hepatocytes in primary culture

5. References

1) Pitot H.C., Goldsworthy T., Campbell H.A., and Poland A. Quantitative evaluation of the promotion by 2,3,7,8-tetrachlorodibenzo-p-dioxin of hepatocarcinogenesis from diethylnitrosamine. Carcinogenesis 8, 1491-1499

2) Schrenk D., Buchmann A., Dietz K., Lipp H.-P., Brunner H., Sirma H., Münzel P., Hagenmaier H., Gebhardt R., and Bock K.W. (1994): Promotion of preneoplastic foci in rat liver with 2,3,7,8-tetrachlorodibenzo-p-dioxin, 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin, and a defined mixture of 49 polychlorinated dibenzo-p-dioxins. Carcinogenesis 15, 509-515

3) Poland A. and Knutson J.C. (1982): 2,3,7,8-Tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: Examination of the mechanism of toxicity. Annu. Rev. Pharmacol. Toxicol. 22, 517-554

4) Poellinger L., Göttlicher M., and Gustafsson J.-Å. (1992): The dioxin and peroxisome proliferator-activated receptors: nuclear receptors in search of endogenous ligands. Trends Pharm. Sci. 13, 241-245

5) Schrenk D., Lipp H.-P., Wiesmüller T., Hagenmaier H., and Bock K.W. (1991): Assessment of biological activities of mixtures of polychlorinated dibenzo-p-dioxins: Comparison between defined mixtures and their constituents. Arch. Toxicol. 65, 114-118

6) Cuthill S., Wilhelmsson A., and Poellinger, L. (1991): Role of the ligand in intracellular receptor function: receptor affinity determines activation in vitro of the latent dioxin receptor to a DNA-binding form. Mol. Cell. Biol. 11, 401-411

7) Birnbaum L.S (1986): Distribution and excretion of 2,3,7,8-tetrachlorodibenzo-p-dioxin in congenic strains of mice which differ at the Ah locus. Drug Metab. Dispos. 14, 34-40