Time- and dose-related effects of TCDD on retinoid parameters in rat tissues

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Introduction

It has long been known that normal retinoid metabolism is perturbed by environmental contaminants such as chlorinated hydrocarbons. Administering 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to rats inhibits normal storing of vitamin A in the liver, while kidney levels increase (1). We have previously shown that seven days after administering a single oral dose of 10 µg TCDD/kg bw to rats, LRAT activity is virtually obliterated in hepatic stellate cells (SC), which is where the large majority of the liver vitamin A is found, mainly in the form of retinyl esters (2). In addition, kidney LRAT activity increases in TCDD-treated rats (2). Thus, in TCDD-treated rats, hepatic and SC retinyl ester levels decreases along with SC LRAT activity, while renal retinyl ester levels and LRAT activity both increase. The present study was designed to further investigate the effects of TCDD on retinoid homeostasis, such as the time- and dose-dependent effects on retinol esterification in liver and kidney, but also looking at levels of serum retinoids and at levels of serum retinol-binding protein (RBP).

Methods

Animals and study design

Male Sprague-Dawley rats had free access to diet (see below) and water at all times.

Time course study: During the one week acclimatization period, and throughout the study, the rats had free access to R34 diet (Lactamin, Stockholm, Sweden), stated to contain 12 000 IU vitamin A per kg. The rats weighed 239 ± 12 g at the time of administration. They were given a single oral dose of 0 or 10 µg TCDD/kg bw, in a vehicle of corn oil. At each time point (1, 3, 7, 28, 56 and 112 days after administration), six control and six TCDD-treated rats were sacrificed, selected organs were excised, weighed and frozen in liquid nitrogen.

Dose response study: From weaning, during a two month period and throughout the study, the rats had free access to R34 diet (Lactamin, Stockholm, Sweden), formulated to contain 4 000 IU per kg. The rats weighed 418 ± 23 g at the time of administration. Groups of six rats were given a single oral dose of 0, 0.1, 1.0, 10, or 100 µg TCDD/kg bw, in a vehicle of corn oil. Three days after administration, all rats were sacrificed as described above.

Biochemical analyses

Retinoids in liver and kidney homogenates were extracted using diisopropyl ether essentially

according to van der Berg et al. (3). Serum retinol and retinoic acids were determined with the gradient HPLC system described by Wyss and Bücheli (4).
Liver and kidney homogenates were assayed for retinol esterification as previously described (1,5). Free [^{3}H]retinol was used as substrate, and the final concentration (10 μM) was shown to be saturating for the LRAT activity (data not shown). Phenylmethylsulfonyl fluoride (1.6 mM) was used as an LRAT inhibitor.
Retinol binding protein (RBP) was measured in serum, liver, and kidney using a radioimmunoassay (RIA) method that has previously been described (6). RBP levels were analyzed in the time study only.
Values are expressed as arithmetic mean ± one standard deviation for individual groups of animals. A significance level of $p<0.05$ was chosen. Student's *t*-test (time study) and One-Way ANOVA (dose response study) were used for statistical analyses.

Results

In the time course study, control rats steadily increased their hepatic retinoid levels over time, while in the TCDD-treated rats, liver levels were virtually unchanged for 28 days, after which liver retinoids began to increase (Figure 1). Renal retinoid levels in TCDD-treated rats were increased, beginning three days after TCDD administration (Figure 2). Serum retinol levels were increased after seven days (a tendency to increase was visible after three days)(Figure 3). Serum retinoic acid levels were increased beginning 24 hours after administration (Figure 4). In the dose response study (data not shown), hepatic retinoid levels tended to decrease in the 1.0 and 10 μg/kg dose groups three days after administration, and were significantly decreased in the highest dose group. Renal levels tended to increase in the 1.0 and 10 μg/kg dose groups, and were significantly increased in the highest dose group. Serum retinoic acid levels were dose-dependently increased.

In the time course study, liver LRAT activities in TCDD-treated rats were not significantly different than control activities (data not shown). Renal LRAT activities were maximally increased (approximately 3 times) already 24 hours after administration (Figure 5). In the dose response study (data not shown), hepatic LRAT activities were unaffected by TCDD treatment. Renal LRAT activities increased in a dose-dependent manner.

In the time course study, TCDD caused increased RBP protein levels in the liver during the first half of the study (Figure 6). In serum, both control and TCDD rats showed a transient increase in RBP levels (data not shown). The increases were statistically significant at days one and seven. Renal RBP levels were unaffected by TCDD treatment (data not shown).

Discussion

The increased renal retinyl ester levels are most likely due to the increased renal LRAT-catalyzed retinol esterification. The effects followed similar time curves, with the increased LRAT activity preceding the increased retinyl ester levels. The increased renal retinol esterification seen following TCDD treatment may be an attempt to salvage the large amounts of vitamin A being filtered through the kidney in TCDD-treated rats (measured as increased urinary excretion [7]), or may be a "normal" response to an abnormal physiological signal.

The full significance, as well as the origin, of the increased serum levels of RA in TCDD-treated

Figure 1. Hepatic retinoid levels

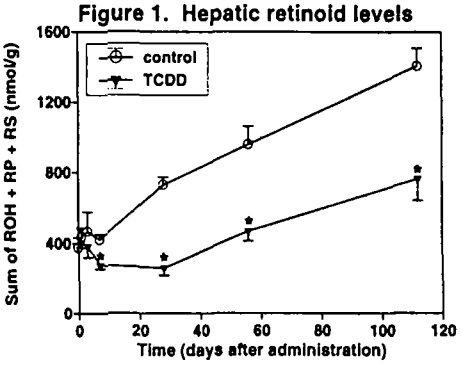


Figure 2. Renal retinoid levels

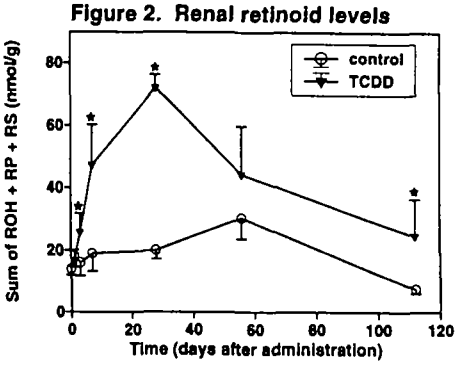


Figure 3. Serum retinol levels

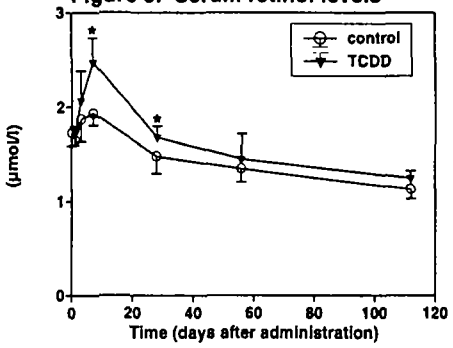


Figure 4. Serum retinoic acid levels

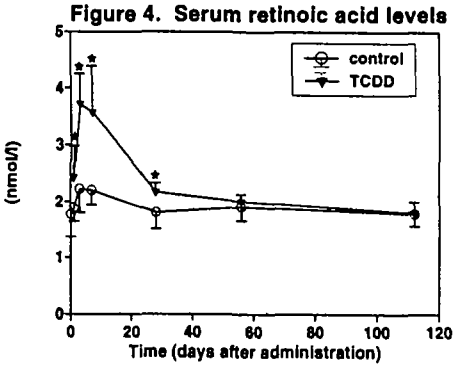


Figure 5. Renal LRAT activity

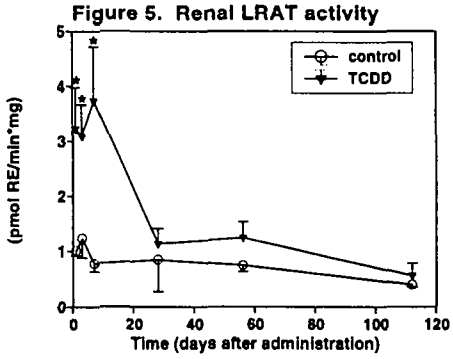
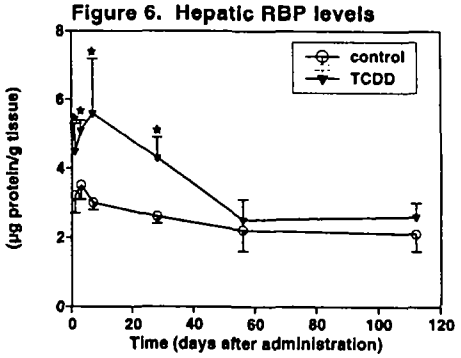


Figure 6. Hepatic RBP levels



rats is unclear. Even in untreated rats, the ultimate role of circulation-derived RA in the tissues remains to be investigated (8). However, a doubling of the serum level of this active retinoid metabolite is likely to have an impact on tissue retinoid homeostasis. Since the blood is filtered by the kidney, it is possible that increased RA serum levels are filtered, and thus found in kidney cells, and thereby causing the increased renal LRAT activity. Published data suggest that the LRAT gene has got a RARE in its promotor (9, 10).

Hepatic protein levels of RBP were rapidly increased in rats given a dose of 10 μg TCDD/kg. A similar effect is seen in vitamin A-deficient rats, in which the mRNA levels are unaffected (11), and it is believed that it is a lack of ligand (ROH) that hinders the excretion of the RBP-ROH-TTR complex from the livers of vitamin A-deficient rats. Hepatic mRNA RBP levels in TCDD-treated rats have not yet been investigated.

In conclusion, results presented here imply an involvement of several tissues in the disturbed retinoid homeostasis seen in TCDD-treated rats. Certain effects such as increased renal LRAT activities and increased serum retinoic acid levels stand out as early, sensitive parameters that may provide important clues to the mechanism behind the effect of TCDD on retinoid homeostasis.

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