TCDD-induced changes in liver phospholipids and cholesterol of Long-Evans Rat

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INTRODUCTION

TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin is a nearly water insoluble, lipophilic compound, the administration of which may result in a delayed appearance of mortality after impressive loss of body weight. This "wasting syndrome" is associated with a wide variety of alterations *eg.* in histopathology, hematology, endocrinology, neurobehaviour, neurochemistry, immune system (for review, see ref. 1), and in body lipids. The latter include mobilization of lipid stores from the adipose tissue resulting in increases in serum lipids (2-4) and an accumulation of lipids in the liver (5). In addition, TCDD has been shown to elevate serum cholesterol and affect the composition of cellular membranes (for review, see ref. 1). However, interspecies, interstrain, and even substrain differences in the susceptibility to the lethal action of TCDD are wide, and in rats the oral LD_{50} values vary from 9.8-17.7 µg/kg for L-E rats up to > 9 600 µg/kg for H/W rats (1). In this paper, changes occurring in membrane phospholipids and cholesterol of TCDD-susceptible Long-Evans rats are reported after sublethal (5 µg/kg) and lethal (50 µg/kg) doses of TCDD.

Materials and Methods

A total of 66 adult, 19 to 21 week-old, female Long Evans rats were assigned to groups of 6 rats and given a dose of 5 or 50 µg/kg TCDD by gavage dissolved in corn oil at 4 ml/kg. The ad libitum-fed control rats and feed-restricted control rats for 4 and 8 days groups received pure corn oil. The feed restricted controls were given feed according to the amount known from previous

experiments to be consumed by 50 µg/kg TCDD-treated rats, after correction for their metabolic body weight (body weight$^{0.75}$). The rats were killed by decapitation one, four and eight days after the exposure. Livers were quickly removed, weighed and frozen in liquid nitrogen, and stored at -80 °C until the lipid analysis.

For the lipid analyses, liver samples of less than 100 mg (wet wt) were solubilized in 5 ml of chloroform / methanol, 2:1 (v/v), homogenized by Potter-Elvehjelm glass-teflon homogenizer and extracted according to the method of Folch *et al.* (6) as described previously (7). The homogenized liver was separated by centrifugation, and the precipitate was reextracted with 5 ml of chloroform / methanol, 2:1 (v/v). The solvents (10 ml) were pooled, 2 ml of 0.88 % NaCl was added, shaken, and two phases were allowed to separate. The lower organic phase was collected, and dried in a vacuum centrifuge. Total lipid extracts were streaked on thin layer chromatography (TLC) silica gel G plates (Macherey-Nagel), and developed in petroleum spirit / diethylether / acetic acid, 80:30:1 (v/v) as presented previously (8). Phospholipids (PL) and cholesterol esters (CE) were detected under a ultraviolet light after spraying with rhodamine 6GO (0.01 % w/v; Chroma), and identified by comparing their R_f values to those of standards (Sigma). Lipid spots were removed, an internal standard (heptadecanoate, Sigma) was added, and fatty acids were analyzed by gas-liquid chromatography as described (9). Extractions and storage of lipid samples occurred under a nitrogen atmosphere. Total sterols (free and esterified cholesterol) were extracted three times with 3 ml of hexane after the addition of an internal standard (ergosterol), and the hydrolysis of the lyophilized liver samples (< 100 mg) in 3 ml of 3.7 M NaOH in 49 % methanol (9). The combined hexane extracts were washed with 2 vol of 0.3 M NaOH, dried with anhydrous Na_2SO_4, and cholesterol was analysed by using Hewlett Packards HP G1800A GCD System. The GLC conditions were as presented: HP-5 capillary column (30 m, 0.32 mm, 0.25μm); carrier gas helium; injector and detector temperatures 325 °C; oven temperature was programmed to hold 175 °C for 1 min, and then increase 10 °C/min to 300 °C. All the values are the means of six independent samples.

Results and discussion

After the lethal dose of TCDD (50 µg/kg), the liver PL content increased from 95.7 ± 11.4 mg/g dry wt of the first day after the exposure to 151.4 ± 15.1 mg/g dry wt present on day eight. In ad libitum-fed controls (91.7 ± 7.9 to 106.9 ± 17.4 % of dry wt), feed-restricted controls (98.2 ± 11.2 to 111.1 ± 16.4 % of dry wt), and in rats treated with a non-lethal dose of TCDD (5 µg/kg) (88.6 ± 7.9 to 108.7 ± 15.9 % of dry wt) minor alterations were observed in the membrane PL contents of the livers during the experiment. Concomitantly, alterations in the membrane-bound cholesterol content (ad libitum-fed control, 6.7 ± 1.3 to 9.3 ± 2.1; pair-fed control, 3.9 ± 2.0 to 7.3 ± 2.1; TCDD 5µg/kg, 7.1 ± 2.2 to 9.0 ± 3.4; TCDD 50 µg/kg, 5.9 ± 2.2 to 10.4 ± 2.3 µg/kg) were not

statistically significant. However, since the membrane PL content increased heavily after the lethal TCDD dose the ratio of PL to cholesterol increased from value 11.5 of the first day to 11.9 of the fourth day and to 25.7 of the eight day, that is the relative amount of cholesterol decreased in the membrane concomitantly with the increase in the proportion of PL bound stearic acid and decrease in that of arachidonic acid (Fig. 1). The CE content increased between days one and eight from 5.6 ± 1.4 to 16.1 ± 2.7 mg/g dry wt after the lethal TCDD dose (50 μg/kg), and upto 15.5 ± 3.8 in pair-fed control. As a result, the total cholesterol content did not alter in ad libitum-fed control (10.0 ± 1.1 to 12.8 ± 2.0 mg/g dry wt), and after the TCDD dose of 5 μg/kg (10.3 ± 1.5 to 12.2 ± 3.3), but was slightly elevated in pair-fed control (12.8 ± 0.6 to 13.9 ± 1.6 mg/g dry wt; days four and eight), and increased after the lethal TCDD dose from 11.6 ± 1.0 to 15.2 ± 2.2 mg/g dry wt when the time passed from the first to the fourth day.

The results showed that after the lethal TCDD dose remarkable changes occur in liver phospholipids and their fatty acids, and in the membrane-bound cholesterol content. The CE content increased both in feed-restricted control and after the lethal TCDD dose. However, after the lethal TCDD dose L-E rats were not able to increase the content of memrane-bound cholesterol at the same rate as the PL biosynthesis increased. The membrane rigidifying effect of cholesterol decreased, which seemed to be compensated with the increase in the highest melting-point fatty acid, stearic acid. These changes in membrane PL fatty acids and cholesterol may be of relevance in the sensitivity of L-E rats to TCDD, known often to end to death because of bleeding.

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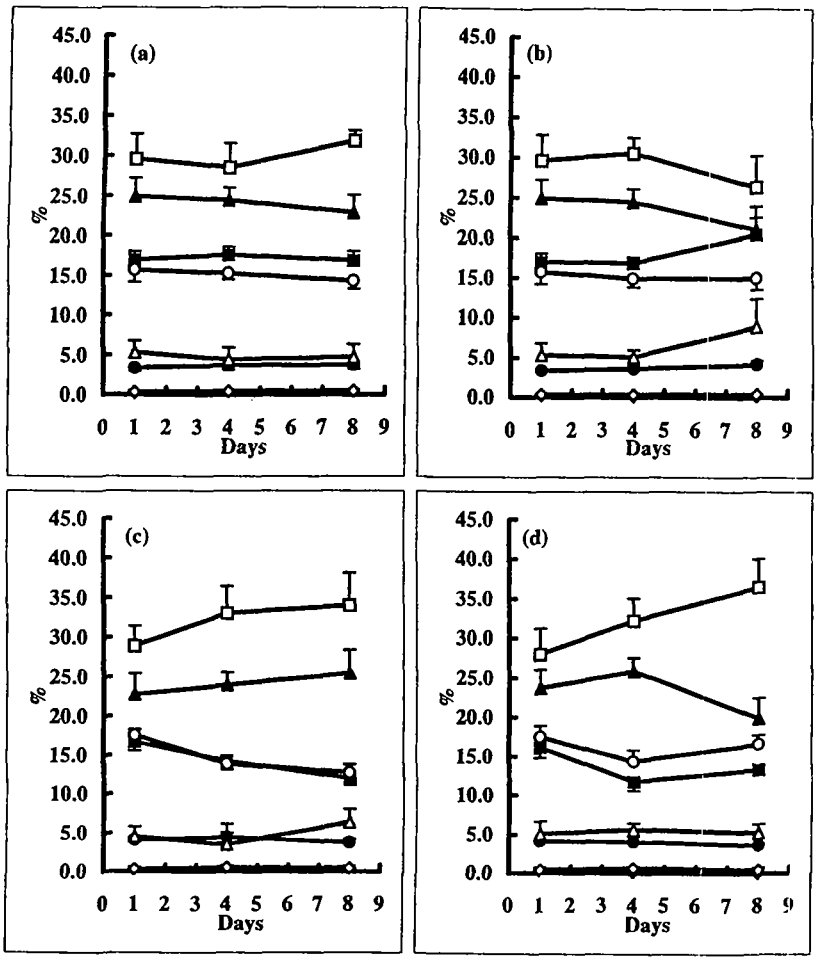

Figure 1. Effects of sublethal (5 μg/kg) (c) and lethal (50 μg/kg) (d) doses of TCDD on liver phospholipid fatty acids of Long-Evans rats in comparison to ad libitum (a) and pair-fed (b) controls (palmitic acid, ■; stearic acid, □; oleic acid, ●; linoleic acid, ○; arachidonic acid, ▲; eicosapentaenoic acid, ◆; docosapentaenoic acid, ◇; docosahexaenoic acid, △).