Amelioration of Short-term Toxicity of TCDD with a Fish Oil Rich in ω-3 Fatty Acids in Long-Evans Rats

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1. Introduction

A single administration of a lethal dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) causes a wasting syndrome in most species or strains of laboratory animals. Over 2-3 weeks, animals may lose up to 50% their body weight while they progressively diminish their eating to a level that is not compatible with life[1]. The mechanisms of this peculiar syndrome has remained elusive.

Although not identical, we have reported remarkable similarities in the clinical and biochemical manifestations of TCDD-induced wasting syndrome and malignant tumor-induced cachexia. These include the progressive weight loss with death at the terminal stage, peripheral lipolysis and elevated serotonergic tone in the central nervous system [2), 3), 4), 5)]

It has been reported that in the experimental cancer cachexia model, the enrichment of ω-3 fatty acids in the diet of tumor-bearing mice with fish oil inhibits the tumor-induced weight loss[6]. Therefore, in the present study we have tested whether the same fish oil with a high content of ω-3 fatty acids might affect TCDD wasting syndrome and biochemical manifestations of its acute toxicity.

2. Materials and methods

Animal husbandry. Adult male Long-Evans (Turku/AB) rats were obtained from the breeding colony of the National Public Health Institute, Kuopio, Finland. They were kept in stainless steel wire-bottom cages equipped with feeding tunnels and cups enabling accurate measurement of food intake and food spillage. During the experiments, the rats had free access to regular feed (R36, Ewos, Södertälje, Sweden) and tap water. The relative humidity in the animal room was 55±10 %, temperature 21.5±1 °C and 12/12 hr light/dark rhythm with lights on at 0700.

Experimental design. Two separate experiments were made. In the first one we tested if the ω-3 fatty acid - supplemented diet might affect the wasting syndrome and lethality induced by a lethal dose of TCDD. For this purpose, 24 rats were treated either with a lethal dose of TCDD (20 μg/kg, intraperitoneally; >LD50[1]), or the same volume of the solvent (corn oil, 5 ml/kg). Six days previously, a treatment regimen had begun where half the rats of both TCDD and control groups were given either a fish oil with 18% of polyunsaturated ω-3 fatty acids (eicosapentanoic acid; EPA) 5 ml/kg twice daily (during weekends, the rats were given the oil only once at 7,5 ml/kg). The fish oil (Q. P. Corporation, Shibya, Japan) was purchased from Siber Hegner GMBH, Hamburg, Germany and it was stored at 4 °C under nitrogen to prevent oxidation. The other half of the rats were

given the same volumes of corn oil. The rats were monitored daily for lethality, feed intake and body weight (every two days) for 42 days.

In the second experiment we observed the effect of EPA-oil on some biochemical changes associated with TCDD toxicity. For this purpose, rats were given TCDD (20 μg/kg) or vehicle. 2 days earlier, the rats were started a regimen with 5 ml/kg b.i.d. EPA oil, corn oil or no oil at all. The daily feed intake and body weight (every two days) were measured. On day 10, the rats were decapitated and the brain, liver and trunk blood were sampled. Plasma was separated from the blood and all the samples were frozen at -70 °C until analysis.

Biochemical analyses. Brain serotonin (5-HT), its main metabolite 5-hydroxy-indoleacetic acid (5-HIAA), the 5-HT precursor amino acid tryptophan and plasma free and total tryptophan were measured with HPLC as described earlier[2]. Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were measured according to Committee of Enzymes[7].

3. Results

In TCDD-corn oil treated rats, the daily food intake and body weight gain was dramatically and progressively decreased. In contrast, in rats supplemented with EPA-oil, the reductions were not that deep (Fig 1). While in the TCDD-corn oil treated group daily food intake decreased almost to zero within 7-8 days after the exposure, in the TCDD-EPA oil - treated group it always remained at over 4 to 5 grams per day. Comparable trends seemed to occur in body weights in these groups. Oil treatments alone did not markedly affect body weight of the rats. One rat in control-EPA group died on day 14 due to dosing failure.

All the rats in the TCDD-corn oil group died with a mean time to death of 17.2 days. In the TCDD-EPA oil group mortality amounted to only 50 % and death took place later compared with the TCDD-corn oil group (mean time to death 28.6 days).

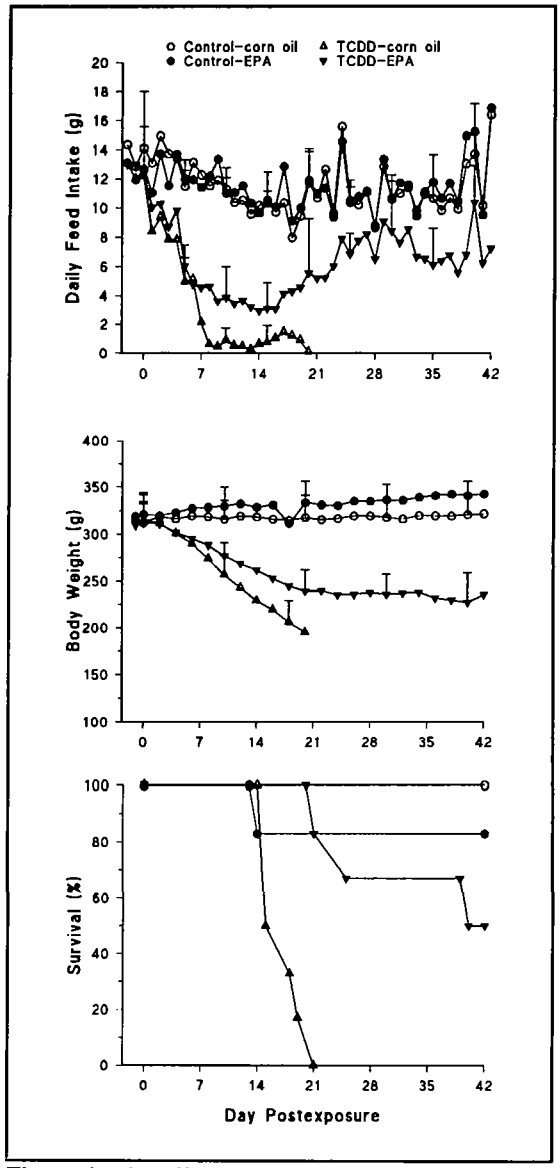

Figure 1. The effect of EPA-oil supplementation on the wasting syndrome induced by a lethal dose of TCDD. N=6.

In TCDD-treated rats there were typical biochemical changes including elevated 5-HIAA and tryptophan concentrations in the brain. In both oil-treated groups, these elevations were abolished.

TABLE 1. Effect of corn or EPA oils on TCDD-induced biochemical changes on day 10

	No oil		Corn oil		EPA oil	
	Control	TCDD	Control	TCDD	Control	TCDD
Brain						
5-HT[1]	3.58+0.18	3.87+0.38	4.15+0.27	3.68+0.21*	4.05+0.51	4.15+0.25
5-HIAA[1]	2.48+0.13	3.20+0.32*	2.73+0.27	2.75+0.22	2.72+0.32	2.69+0.24
Trp[1]	26.4+1.59	35.2+4.28*	28.7+2.41	28.8+1.78	27.3+3.29	30.1+2.82
Plasma						
free Trp[2]	3.50+0.34	8.0+1.47*	3.32+0.42	4.59+0.74	2.86+0.38	4.01+0.85*
total Trp[2]	29.2+4.5	39.6+2.1*	27.1+2.8	26.8+3.2	23.6+5.5	26.0+7.8
ASAT[3]	183+34	1984+2147	241+146	467+217	174+112	254+14
ALAT[3]	50+3.1	119+83	48+8.5	48+12	43+11	30+4.2

Mean±SD. Trp (tryptophan). Statistically significant difference ($p > 0.05$) vs TCDD control is denoted by an asterisk (Student's t-test for independent comparisons). N=3-6. ASAT or ALAT values were not compared for statistical significance. [1] nmol/g tissue, [2] mg/l, [3] activity, U/l.

Plasma free tryptophan was previously shown to correlate well with the severity of the wasting syndrome elicited by TCDD in rats[2), 3)]. Here, plasma free tryptophan concentration was elevated over 2-fold by TCDD alone. If oil was administered, the elevation in free tryptophan was largely antagonized. Interestingly, plasma ASAT activity was extremely high in the TCDD-no oil group. However, number of observation was only 3 in this group and there was a great variation. However, both oils seemed to inhibit this elevation.

4. Discussion

This study revealed protective effects of ω-3 series polyunsaturated fatty acid, eicosapentanoic acid, against TCDD acute toxicity. The hypophagia and consequent body weight loss as normally seen in TCDD-treated rats were attenuated in response to EPA-oil administration. Furthermore, lethality of TCDD was postponed and reduced at least at the TCDD dose employed. However, it is obvious that if larger doses of TCDD had been used, the ability of EPA-oil to prevent TCDD lethality might have been overridden.

These results are analogous to those obtained in cancer models of cachexia. Wasting syndrome in tumor-bearing mice was also inhibited by the same fish oil as used in the present study[6)]. It was shown, that EPA, but not other even closely related fatty acids, was effective in reducing lipolysis induced by MAC16 tumor cell line by

preventing cAMP accumulation in response to lipolytic stimuli [8].

The biochemical parameters we have previously suggested to be closely associated with TCDD wasting syndrome (elevated plasma free tryptophan and consequent increase in brain 5-HT metabolism) were abolished or attenuated with both oil supplementations. Still the anorexia, hypophagia and lethality were postponed only with EPA oil. These findings further support the view that although wasting syndrome and altered tryptophan binding to albumin (increase in free tryptophan) in response to lethal TCDD dose coincide, these two effects do not have to be in causal relationship.

The corn-oil treated rats died sooner after TCDD administration than could have been expected for the dose of TCDD[1]. Therefore, corn-oil seemed to aggravate TCDD toxicity whereas EPA-oil clearly attenuated it. Corn oil contains mainly (ca. 60%) linoleic acid. The crucial biochemical difference between linoleic and EPA lies in the fact that linoleic acid promotes arachidonic acid metabolism and EPA diminishes it[9]. Thus, the present findings implicate arachidonic acid and its derivatives (eicosanoids) in the acute toxicity of TCDD.

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