Alteration of Cellular Utilization of Thymidine by TCDD (2,3,7,8-Tetrachlorodibenzo-*p*-dioxin)

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Polychlorinated dibenzo-p-dioxins are found among the commercial products of chlorinated phenols such as 2,4,5-T as contaminants. Many of these contaminants are highly biologically active causing teratogenic effects, mortality, and skin problems such as chloroacne. Among them 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is acknowledged to be the most potent bioactive member. Its LD_{50} against the guinea pig has been cited at 0.6 μg/kg (SCHWETZ et al. 1973). Its toxicity manifests in a peculiar manner in that the animal receiving a low dose of TCDD gradually loses weight, and death occurs after a long latent period. For instance VOS et al. (1973) report that all guinea pigs treated at the 1 μg/kg per week for 8 weeks level died between 24 and 32 days (mean 28 days) after the treatment. The most significant organ weight loss occurred in the thymus, with lesser weight loss in the liver. Its effect appears to be accumulative as shown by the fact that chronic daily dosing begins to manifest its effect when the cumulative dose reaches the same level as that of the effective single dose (ALLEN and CARSTENS 1967, HARRIS et al. 1973). In the rat gross pathological changes are observed in the thymus and liver at a single dose of 50 μg/kg which resulted in 7% mortality for males and females (GUPTA et al. 1973). While the mode of action of TCDD to cause such pathological lesions has not been elucidated, it is clear that TCDD at low doses causes changes in various liver functions through apparent "induction" actions (BUU-HOI et al. 1972, GRIEG 1972, FOWLER et al. 1973, LUCIER et al. 1973) with increases in P450, cytochrome b_5 and microsomal protein. It does not, however, induce δ-aminolevulenic acid synthetase in adult rat liver (POLAND and GLOVER 1973). Our experiments were conducted to determine if TCDD affected the utilization and intracellular distribution of precursors of RNA and DNA in rat liver.

MATERIALS AND METHODS

Male rats 130-150 g (Sprague-Dawley, Madison, Wisconsin) were used in all experiments; a single dose of 5 μg/kg TCDD dissolved in 1:9 acetone-corn oil was administered by a stomach tube method under ether anaesthesia. Control rats were treated identically without TCDD. All the rats were then fed a normal Purina rat chow and water diet ad libitum for a period of ten days.

After the 10-day post administration period, the rats were killed by cervical dislocation, and the livers were excised and

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rinsed in 0.9% NaCl at 37°C. Slices 1 mm thick and weighing 40-60 mg were prepared from the left lateral lobe by using a McIlwain tissue chopper.

All incubations were carried out in Swim's-S-77 medium at 37°C with 10% inactivated fetal calf serum (FCS) supplemented with 200 mM glutamine and 10 µl/ml antimycotic mixture (Grand Island Biological Co., Grand Island, New York). After the initial temperature equilibration period for 3 minutes in unlabeled medium, the slices were placed in 10 ml of medium containing 0.5 µCi of thymidine-methyl-^{3}H, or 1 µCi of uridine-5-^{3}H (Amersham-Searle, Clearbrook, Ill.). The slices were pulse-labeled for 16 minutes, blotted dry on filter paper, and rinsed twice in 10 ml of medium with blotting after each rinse. In the subcellular distribution studies, 25 slices of pulse-labeled liver were incubated in 25 ml of medium with shaking for 1 hour in a 95% Air-5% CO_2 atmosphere. At the end of the experiment, the slices were blotted on filter paper and rinsed twice in .25 M sucrose, and homogenized with ten strokes of a teflon pestle in a Potter-Elvehjem homogenizer. The subcellular distribution of radioactivity was measured by differential centrifugation: nuclei-700 x g for 10 min., mitochondria-7,000 x g for 20 minutes, and microsomes-105,000 x g for 1 hour. The nuclear and mitochondrial fractions were rinsed once with 3 ml .25M sucrose, and re-isolated by centrifugation. Prior to scintillation counting, .2 ml of each fraction was placed in a 20 ml scintillation vial with a .5 ml aliquot of NCS solubilizer (Amersham-Searle). The vial was then heated for 4 hrs at 50°C to solubilize the proteins. Protein determinations in all samples were carried out by using the method of LOWRY et al. (1951).

Studies on isolated nuclei were performed on free nuclei prepared by the method of BLOBEL and POTTER (1966), with the exception that 2.1 M sucrose was used in the standard 0.05 M tris-HCl buffer (TKM), (BLANKENSHIP and KUEHL 1973), and that the homogenate was not filtered. Nuclear membranes were separated from the nuclear chromatin material by the method of BORNENS (1973), using 10 mg/ml heparin. Radioactivity in the chromatin fraction was determined by subtracting the radioactivity in each membrane fraction from the total radioactivity of the original aliquot of suspended nuclei. In identical experiments with unlabeled nuclei the membrane fraction was determined to contain 18.5 to 19% of the total nuclear protein.

RESULTS AND DISCUSSION

Microsomal function, as judged by their epoxidation activities on aldrin, and protein synthesis, from evidence of ^{14}C-phenylalanine incorporation in the microsomal fraction, remained unaffected in liver slices for at least 3 hours under the experimental conditions. It was also shown that the rate of thymidine or uridine uptake by the slices reached a plateau at 16 minutes. The slices were thus pulse-labeled for 16 minutes, and then incubated further in the nonradioactive medium for an additional 1 hour. Upon

fractionation of intracellular components it became clear that the incorporation of thymidine-^{3}H is significantly increased in nuclei from TCDD-treated rats (Table 1), with corresponding decreases in the supernatant fraction, while that of uridine-^{3}H was unaffected.

TABLE 1

Incorporation of ^{3}H-thymidine and ^{3}H-uridine in subcellular fractions of incubated liver slices

	^{3}H-thymidine incorporation[a]			
	control (4 rats)		dioxin-treated (5 rats)	
	cpm%	cpm/mg	cpm%	cpm/mg
homogenate	100	79.4 ± 28.0	100	67.0 ± 17.6
nuclei	12.5 ± 3.8	28.8 ± 4.0	24.6 ± 7.4*	44.9 ± 13.2**
mitchondria	7.3 ± 2.5	20.4 ± 3.0	10.1 ± 3.1	17.4 ± 11.1
microsomes	4.0 ± 1.3	16.4 ± 4.8	4.6 ± 0.9	15.0 ± 1.7
supernatant	76.2 ± 6.2	228 ± 114	60.6 ± 9.8	147 ± 71.8
recovery (calculated)	107 ±10.0	73.1 ± 18.9[c]	115 ±10.5	58.1 ± 15.8[c]
	^{3}H-uridine incorporation[b]			
	control (2 rats)		dioxin-treated (5 rats)	
	cpm%	cpm/mg	cpm%	cpm/mg
homogenate	100	150 ± 67	100	171 ± 16
nuclei	6.2 ± 1.6	24 ± 10	6.4 ± 1.0	37 ± 11
mitchondria	4.1 ± 2.2	21 ± 6	3.9 ± 1.1	23 ± 5
microsomes	2.9 ± 0.8	53 ± 14	3.4 ± 3.5	42 ± 32
supernatant	87.0 ± 1.5	997 ± 344	88.7 ± 7.5	1028 ± 228
recovery (calculated)	105 ± 11.6	172 ± 52[c]	108 ± 4.7	214 ± 30[c]

[a] ^{3}H-thymidine specific activity 22.4 Ci/m Mole (counting efficiency 20.2%)

[b] ^{3}H-uridine specific activity 20.0 Ci/m Mole

[c] Average total counts in cell fractions/Average total mg protein in cell fractions.

* $t = 2.94$ significant at 95% level ($P < 0.05$)

** $t = 2.33$; $t_{.05} = 2.365$

The differences in the incorporation of thymidine-^{3}H was verified by analysis of the purified nuclei obtained by the Blobel-Potter method, (Table 2). To examine the intranuclear site which could account for the difference, the nuclear membrane was separated from the chromatin material. It was calculated from the total radioactivity in each nuclear fraction that a large portion of radioactivity (51.3% in control and 78.2% in TCDD-treated) was associated with the chromatin material, and that the difference was more pronounced in this fraction, indicating that the stimulation of thymidine-methyl-^{3}H incorporation by TCDD is an intranuclear phenomenon (Table 2).

TABLE 2

^{3}H-thymidine in isolated and purified nuclear components

	cpm/mg protein[a]	
	untreated	TCDD-treated
purified nuclei	30.5 ± 25.5 (7 rats)	59.1 ± 24.0 (9 rats)*
nuclear membrane	37.4 (3 rats)	50.4 (3 rats)
isolated chromatin	17.1 (3 rats)	43.7 (3 rats)

[a] ± standard deviation

* $t = 2.30$ significant at 95% level ($P < 0.05$)

If one accepts that the above disruption phenomenon on nuclear utilization of thymidine means the direct interference of TCDD on the process of DNA synthesis, there is information that is mutually compatible in explaining the mechanism of the action of TCDD on living cells.

JACKSON (1972), for instance, demonstrated that TCDD in doses as low as 0.2 µg/l severely disrupts the mitotic activity of dividing endosperm cells of the African blood lily with the formation of dicentric bridges and chromatin fusion. VOS et al. (1973) concluded that TCDD at sublethal doses suppressed the cell mediated immunity as well as the total number of leukocytes and lymphocytes formed in the guinea pig. Moreover, such an action on DNA could conveniently explain TCDD's teratogenic properties (SCHWETZ et al. 1973, SPARSCHU et al. 1971) as well as the high embryotoxicity and embryomortality (NEUBERT et al. 1973). It is noteworthy that such a significant effect on thymidine utilization by the nucleus takes place at 5 µg/kg vs. estimated LD_{50} of 22 µg/kg in the adult male rat (SCHWETZ et al. 1973), even after 10 days from the date of administration. While the data presented here is not sufficient to conclude a specific mechanism of action of TCDD in terms of the actual process of interference with thymidine utilization, the phenomonenon itself is significant enough to warrant special attention in view of the importance of this pesticidal contaminant in Environmental Toxicology.

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