The Testes are a Target for 2,3,7,8-Tetrachlorodibenzodioxin in C57 Mice

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Introduction

A decline in human sperm count and quality in the past decades seems to take place in industrialized countries. The release of persistent organochlorines into the environment, especially of compounds with endocrine disrupting activity, is discussed as an important possible cause. Since the spraying of the herbicide Agent Orange during the Vietnam War 2,3,7,8-Tetrachlorodibenzodioxin (2,3,7,8-TCDD) and other polychlorinated dioxins (PCDD) and furans (PCDF) are under suspicion of affecting male fertility. A prospective study among 872 Vietnam veterans could however not find a significant relation between TCDD serum levels and amount and quality of the sperm collected in 1982. However, a siginificant negative correlation between TCDD serum levels and the size of the testes was found [1]. In three pooled sperm samples from 17 Vietnam veterans relatively low levels of 0.011 - 0.015 pg I-TEQ/g wet weight were found [2]. Further data on dioxin concentrations in sperm from men without occupational exposure are not available. In order to find out whether the testes are a target for dioxins we applicated repeatedly moderate doses of 2,3,7,8-TCDD to male C57 mice and analyzed after a certain time (1) the TCDD tissue concentrations in liver, adipose tissue, and testes, and performed (2) a histological examination of liver, testis, and epididymis.

Material and Methods

Male C57BL/6J mice were treated three times (every 10^{Th} day) s.c. with 5 µl of a 2,3,7,8-TCDD solution in toluene/DMSO (1+2, v/v) equivalent to 16 and 160 ng 2,3,7,8-TCDD per kg body weight, respectively. Seven days after the last injection the mice were sacrificed. Adipose tissue, liver, and one testis from each animal were freeze-dried and extracted with toluene in a soxhlet apparatus. After two clean-up steps the purified extracts were analyzed for their 2,3,7,8-TCDD levels by HRGC/HRMS using $^{13}C_{12}$-labeled 2,3,7,8-TCDD as internal standard. The second testis together with the epididymis of each animal were weighed and histologically examined.

Results and Discussion

No significant changes in body weight or in weight of the testes plus epididymes occurred either for low or high TCDD doses compared to control mice receiving vehicle only. This would suggest that overall exposure to the levels of 2,3,7,8-TCDD used in this study did not produce any gross pathological systemic effect and had no gross effects on these reproductive organs. This

was supported by histological analysis of the testes and epididymes where except for one animal the morphological appearance of these tissues was similar to that seen in control animals.
All concentrations in the following table are in pg/g wet weight (ppt). In both dose groups significant amounts of 2,3,7,8-TCDD were recovered in the testes (0.6 and 0.4 % of the total dose). The concentrations of 22.1 and 163 ppt were equivalent to 18 and 15 % of the corresponding levels in adipose tissue. In the 16 ng/kg group only 5 % of the total dose was recovered in the liver. The levels in adipose tissue were more than twofold higher than in the liver. The application of the tenfold dose led to 20fold higher concentrations in the liver resulting in a hepatic retention of 9.3 %. Both, in adipose tissue and testis the concentrations in the high dose group were about 7.5-fold higher than in the low dose group.

Single dose	Control	16 ng/kg b.w.		160 ng/kg b.w.	
Number of animals	2	3		4	
		mean	SD	mean	SD
Concentration in liver (pg/g)	<1.1	**59.0**	8.9	**1168**	214
Recovery in liver (%)	-	4.9	1.2	9.3	2.1
Concentration in adipose tissue (pg/g)	<7.1	**139**	32	**1058**	230
Concentration ratio liver/adipose tissue (%)	-	44	8	113	24
Concentration in testis (pg/g)	<7.5	**22.1**	12.3	**163**	92
Recovery in testis (%)	-	0.62	0.37	0.40	0.20
Concentration ratio testis/adipose tissue (%)	-	18	13	15	9

The data clearly show that at low and moderate doses the testes of C57 mice are target organs for 2,3,7,8-TCDD. After a 22 week exposure of male rats to very high 2,3,7,8-TCDD doses (initial dose of 25 µg/kg b.w. and a weekly maintenance dose of 5 µg/kg b.w.) [3] found relatively low concentrations of 500 pg/g in the testis which were equivalent to only 2 - 2.5 % of the corresponding levels in adipose tissue as compared to 18 % respectively 15 % in our experiment. In vivo studies with rats have shown that the hepatic retention of TCDD increases with higher doses and the retention in other tissues decreases [4].
Our results contradict the notion that the testis is protected from the deposition of dioxins. Further analyses of persistent organic pollutants with endocrine disrupting activity in human testicular tissue and sperm fluid are warranted. Particularly, since results of *in vitro* studies using tubuli seminiferi from rats have shown that PCBs penetrate the blood-testis barrier and damage spermatogenic function [5].

References

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