STUDY OF LONG - TERM EFFECTS OF TCDD ON THE MICROSOMAL MONOOXYGENASES OF RAT LIVER

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Introduction

By now it is known that the most essential moment in pathogenesis of the intoxication of 2,3,7,8 - tetrachlorodibenzo - p - dioxin (TCDD) is its permeability into cell cytoplasm and its binding to the specific cytosole protein – Ah-receptor. A complex of TCDD – Ah-receptor penetrates to a nucleus, binds with a certain locus of DNA inducing the stimulation of gene expression, coding a structure of cytochrome P450-dependent microsomal monooxygenases. Namely this event follow-up in TCDD intoxication determines the induction of a number of enzymes in mammalian organisms. At present a strong correlation of toxicity of TCDD and similar compounds to their ability to induce microsomal monooxygenases (the products of a family of genes P4501A) has been proved (1-3).

From a practical point of view the data about minimal doses giving the induction of enzymes above and its time duration is known to be very important. According to the results of Kitchin and Woods (4), induction of enzymes in rat organism appeared after a single exposure to TCDD in dose of 0.002 μg/kg body weight (bw). However, according to the data of Masuda et al. (5), a minimal inducing effect was observed after a dose of 0.1 μg TCDD /kg bw. The time-dependent effects of TCDD on microsomal ethoxyresorufin *O*-deethylase (EROD) and methoxyresorufin *O*-demethylase (MROD) activities in the liver of rats exposed to 10μg TCDD /kg was observed (6). Three and seven days after TCDD-treatment, maximal TCDD-induced EROD and MROD activities, respectively, was found, which remained elevated at 35 days after TCDD exposure. According to the data of Kitchin and Woods, TCDD-induced activity of benzpyrene - hydroxylase (BPH) didn't reach a normal level 6 months after a single exposure to toxicant at a dose of 2.0 μg/kg bw. A marked prolonged activation of microsomal monooxygenases is known to be accompanied with an enhanced generation of free radicals and, as consequence, intensification of peroxide processes, suggesting the determination of cytotoxic effects of TCDD in a larger degree.

The data about the status of microsomal monooxygenases under conditions of a subacute and chronic exposure to TCDD is known to be a very interesting thing. However the absence of a clear knowledge about the influence of TCDD on the enzymes of microsomal oxidation and phenomenon of induction duration under conditions of subacute and chronic exposure stipulates for need of the further studies in this direction.

Taking this into account, this research had an aim to study long - term effects of TCDD on the status of microsomal monooxygenases in rat liver under conditions of a subacute exposure.

Material and Methods

Male white mongrel rats with body weights of 150 - 170 g and were fed with a standard animal food and used for the study. The animals were divided into 3 groups: 1 - control; 2 and 3 were animals receiving TCDD in dose of 0.1 and 1.0 μg/kg bw, respectively. TCDD was injected in doses above in 0.2 ml of oliva oil intraperitoneally 5 times a week during a month. Animals' exposure was performed under leadership of Dr. Victor V. Benemansky (Institute of Industrial Medicine and Human Ecology). 6 rats of each group were studied 1, 3, 6 and 11 months after the last injection of xenobiotics.

A microsomal (105000 g) fraction of rat liver homohenate was an object of study. The content of cytochromes b_5 and P450 was measured in microsomes using spectrophotometer Specord M-40/Carl Zeiss, the activity of *N*-demethylase with aminopyrine (APND), *p*-hydroxylase with aniline (AOH) and benzpyrene – hydroxylase on Specol 10/Carl Zeiss with pulleys EKA and FK, respectively (7,8). The choice of BPH is explained so that given response in a case of TCDD is considered to be the best marker reaction to the content of products of gene A1 (1).

TCDD was obtained from Ekros Co. (St. Petersburg, Russia). NADP·H and 3,4 – Benzpyrene were purchased from Sigma Chemical Co. (St. Louis, MO), Natriumdithionit – from Merck (Darmstadt, Germany), all the other chemicals used were of analytical grade.

The study results were statistically processed using Student's criteria.

Results and Discussion

Table 1 shows that dioxin exposure at doses of 0.1 and 1.0 μg/kg bw provoked a marked activation of BPH and increase in the content of cytochrome b_5 in rat liver microsomes. At that a high level of induction was kept during the experiment for 11 months. A clear time-course dependence of the effect expression displaying, first of all, in BPH activation was observed. The data received during 2 last terms of study allowed to judge the dose dependence about what the alterations of values of indices testified. In addition, TCDD exposure gave an activation of aminopyrine *N*-demethylase and aniline *p*-hydroxylase 3 months after the finishing of exposure only a time under conditions above. On this background no alterations in content of a total pool of cytochrome P450 were revealed and no shift of a reduced cytochrome with CO to a short-waved site of spectrum was observed.

On the whole experiment results are concordance with results of many studies concerning TCDD influence on microsomal monooxygenases in liver. In addition, we showed that the BPH induction evoked by TCDD under conditions of 20-fold exposure at doses of 0.1 and 1.0 μg/kg bw had been kept for 11 months. Such conditions may have a place in real extremal situations (for example, during liquidating consequences of accidents, fires and so on). Such a long-term induction of enzymes (11 months for a rat – it is a third of its life) may be one of the evidence of dioxin contact.

It is known that the induction of a family of cytochrome P4501A is accompanied with the formation of high-responsive electrophylic metabolites and active forms of oxygen. In this relation we may note, that we have revealed the intensification of a superoxyde radical generation during the last terms of experiment (6 and 11 months after finishing exposure).

In conclusion it should be noted that the findings about such a long-term and pronounced induction of microsomal monooxygenases are principially very important. Dioxin is known to metabolize extremely slow in human organism ($T_{1|2}$ is about 7 years and more) and this determines the duration of its stay in organism, and hence, an inducing effect on cytochrome P450-dependent monooxygenases during a long-term period. That's why, clearing-up the fact of a pronounced

induction of CYP1A in organism cells may indicate a probability of dioxin contact in the past. Such information together with some investigations, for example, analyses of blood samples and cutaneous fat cells for these compounds was found to be very useful. (3) In this connection we have to give some considerations.

As a result of the examination of a group of chemists – synthesizers at the plant "CHIMPROM" in Ufa, who were exposed to 2, 3, 7, 8 –TCDD during their work, we have found that the content of this isomer in blood ranged between 140 and 1200 pg/g blood lipids at that time (9). At the same time a statistically significant level of the content of PCDDs/PCDFs in blood of healthy donors in city Ufa was 35.6 pg/g blood lipids in 1998 (9). According to the data of A. Schecter, et al. (10) this value was 23 pg/g blood lipids. Furthermore, a number of persons with a very high content of TCDD had no complaints in relation to their health states and clinical manifestations of dioxin intoxication. We have to note that genetic polymorphism and inducibility (ability to induce) of enzymes of biotransformation stipulate for 100 – 1000 fold interindividual differences in their activities (11). Hence, the carries of high and low activity of CYP1A will significantly be differed in the degree of expression of responses to dioxins in effect. So, the assessment of CYP1A activity in persons exposed to dioxins in the past will help to get very important additive information and allow to judge about the correlation between a genotype and pathology, have a very important prognostic value. All the considerations above we have been realizing since January 1999 in the frames of a profound clinical examination of the firemen tooking parts in the liquidation of an accident at the plant "IRKUTSKCABEL" in the city of Shelekhov in December 1992.

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Table 1. TCDD influence on microsomal monooxygenases of rat liver (in % of control)

Animal group, months	Dose, μg/kg	P450	b_5	BPH	APND	AOH
1	0.1	110	122*	329***	103	123
3	0.1	104	118*	257**	113*	117**
6	0.1	90	111**	184***	100	96
	1.0	108	126***	281***	114	109
11	0.1	113	108	136**	113	111
	1.0	109	120**	172**	111	111

* P<0.05, ** P<0.01, *** P<0.001, Student's t-test, n=6.