Different Levels of Hepatic Cytochrome P450 Induction by Equitoxic Quantities of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin Given as a Single or Multiple Doses

Vladimir V. Litvak, Vasily I. Kaledin*, Ludmila F. Gulyaeva**, Eugenia G. Saikovich, Valery P. Nikolin*, Valentin V. Vlassov

Institute of Bioorganic Chemistry, Siberian Branch of Russian Academy of Sciences, 630090 Novosibirsk, Russia.
*Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, 630090 Novosibirsk, Russia.
**Institute of Molecular Pathology and Ecological Biochemistry, Siberian Branch of Russian Academy of Medical Sciences, 630117 Novosibirsk, Russia.

Introduction

The toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is often considered as consequence of hyperinduction of the cytochrome P450 (CYP450) enzyme family[1]. There is a hypothesis, however, that the parallel phosphorylation process of the definite elements of the signal transduction mechanism is the main factor of the toxic action of TCDD[2]. Assuming a first idea about the connection between TCDD toxicity and monooxygenases induction as well as the data[3] of superaccumulation of the TCDD toxic action at the chronic administration, one can suggest that the induction of microsomal enzymes at a single and chronic administration should be the same. We have examined this assumption studying the induction of monooxygenases in the liver of the C57BL mice.

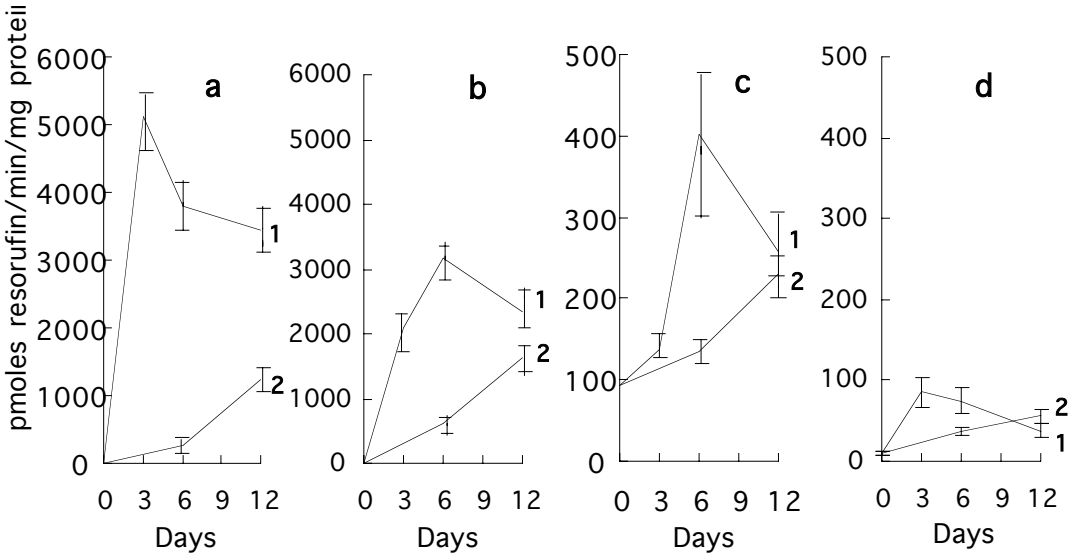
Material and Methods

Inbred C57BL male mice, 3-4 months of age, were obtained from the animal colony of Institute of Cytology and Genetics, Novosibirsk. They were housed in plastic cages (4 mice per cage) and provided with food and water ad libitum. TCDD was synthesized in accordance with Litvak et al.[4]. It was dissolved in olive oil (2 μg/ml, a large dose, and 0.2 μg/ml, a small dose). The solution of TCDD was given to mice by oral gavage 1 ml/kg of body weight singly (large dose) or daily (small dose) within 5 or 10 days (with 48 hours interval between the 5th and 6th intubation in the latter case). There were 4 mice in each group. The mice received large TCDD dose were decapitated 3, 6 and 12 days after dosing; the mice chronically exposed to small dose of TCDD were decapitated 6 and 12 days after the first (24 hours after the last) intubation. The animal's body and liver were weighted. The liver was homogenized on ice and the microsome fraction was separated by differential centrifugation. CYP450 content was determined by the method of Omura and Sato[5], and the activity of enzymes under investigation, by the method of Burke and Mayer[6]. CYP4501A1 and 1A2 induction was assayed by measuring activity of ethoxyresorufin-O-deethylase (EROD) and methoxyresorufin-O-demethylase (MROD) as well as that of CYP4502C and 2B was assayed by measuring activity of pentoxy- and benzyloxyresorufin-O-dealkylases (PROD and BROD, respectively). The protein concentration was determined by the Lowry method. Results are given as mean ± standard deviation, and significance was assessed by the Student's *t*-test.

Fig. 1. Induction of alkoxyresorufin-O-dealkylase activities after administration of TCDD: 1- after single administration of dose 20 μg/kg; 2- after daily administration of dose 0.2 μg/kg. a - EROD; b - MROD; c- BROD; d - PROD.

Results and Discussion

Neither large nor small doses of TCDD caused any visible signs of toxicity in our experiments. Just a slight but valid and similarly expressed body weight loss was observed in all groups of animals. It is not unexpected as the toxic effect of dioxin is known to manifest rather late: even if the dose is hundred times higher than LD_{50}, mice die only in 1.5-2 weeks[3]. The inducing effect of TCDD evaluated both by CYP450 content in liver and the activity of associated monooxygenases (Fig.1) was found in all groups, albeit it was different. The area under the curve in fig.1a illus-



trating the total activity of EROD (the marker of the main inducible by TCDD CYP4501A1) is more than 60 times higher than in the control mice in the case of the experiment with single large dose (see table). It is about 8 times higher than in the control chronic experiment. Thus, we observed that the enzyme could oxidize 7 times greater amount of endogenous equivalents of ethoxyresorufin in the first case than in the second one. However, TCDD toxicity according to Kunzevich[3] is equal in the both cases. Consequently, it is not caused by the hyperinduction of EROD (CYP4501A1). The difference between the levels of induction of BROD and PROD by large and small doses of TCDD are minimal (1.7 - 1.8 times, see Table). These dealkylases also seem to have no bearing on the toxic effect, since they are much greater induced by phenobarbital without any toxic consequences for the organism[7]. The role of CYP4501A2 (marker - MROD) which oxidizes uroporphyrinogene into indigestible uroporphyrin and takes part in iron metabolism, is less clear. The iron loading considerably intensifies the manifestation of TCDD toxicity

The areas under the curves in fig. 1 characterizing cumulative activity of the corresponding enzymes in the course of the experiments (μmoles of resorufin/mg protein per 12 days)

Enzymes	Control	TCDD 20 μg/kg single large dose (A)	TCDD 0,2 μg/kg 10 small doses (B)	A: B

EROD	1.00	61.70 (61.70)*	7.84 (7.84)*	7.87
MROD	1.43	27.67 (19.32)	9.58 (6.69)	2.89
BROD	1.59	4.52 (2.84)	2.55 (1.60)	1.77
PROD	0.18	1.04 (5.74)	0.61 (3.33)	1.72

*In the brackets - fold of induction.

(liver damage, porphyria) in mice[8]. The effect is, however, similarly expressed in mice both with Ah^b and Ah^d receptors[8], so it does not seem to be connected with cytochrome induction. Relatively small toxicity of isosafrol and 1,4-dihydroxyantraquinon which are specific inducers of CYP4501A2[9] supports this conclusion. CYP450 induction at a single TCDD administration is dose dependent to a certain extent and reaches almost maximal values, at dose of 20 μg per of animal weight (rats and mice)[10], when Ah-receptor is saturated by the ligand. When using smaller doses, only a part of receptor molecules is activated and the repeated administrations result in cumulating effect (Fig.1). TCDD toxicity effect (evaluated by animal mortality) follows a different regularity. Judging by data of A.D. Kunzevitch et al.[3], for causing toxic effect, TCDD should be present in animal organisms for at least 10 days. In this case 50% mortality is achieved of TCDD doses which are three orders of magnitude lower than it is required for maximal induction of CYP450. In the chronic experiment, the minimal single TCDD dose should approach this value *d* when daily administered during the given period of time. In the single dose experiment, the given dose *D* should be such as to provide the presence of the *d* dose of dioxin in the organism by the deadline. It was experimentally found that *D* is approximately equal to 1000 *d* [3]. The calculations show that when the daily dose is by half on each successive day over 10 days, the final dose will 1000-fold less than the initial one. Fig. 2 shows calculated content of TCDD in mice after single and repeated administrations assuming the halftime of effectiv TCDD decrease being 24 h. The zero value of lg *d* corresponds to the minimal content of TCDD (approximately 1*d*) which should be kept constant within the whole period of administration to provide 50% lethality. To achieve this effect, it is enough to give 1/100 *D* (10 *d*) of TCDD with the 3.5 days intervals[3] (Fig. 2), or 1/10 *D* (100 *d*) twice with the 7 days interval, or 1/500 *D* (2*d*) 6 times every 40 hours. The experimental support of these predictions would allow us to formulate new approaches to study the mechanism of TCDD toxic effect. In this line, it would be necessary to answer the following questions:

1. What hypotheses are the basis for determination of *d* value: whether there is another, different from Ah-receptor, minor target of TCDD with its own affinity to dioxin, reaction on its effect, reduction time, etc., in different species of animals or TCDD toxicity at low doses is also realized through Ah-receptor triggering the cascade proc ess of phosphorylation of different parts

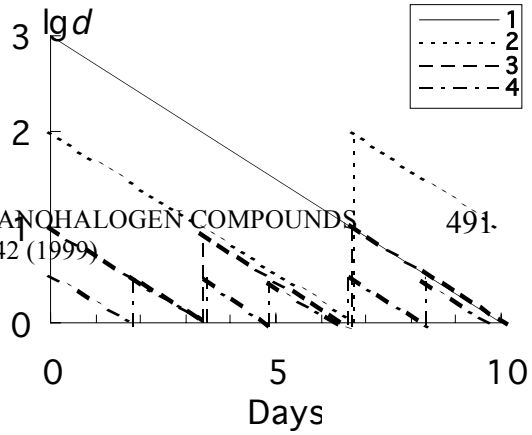


Fig. 2. Periodicity of administration of TCDD needed to achieve 50% lethality of mice according to[3] : 1 - 1000 *d*, 2 - 100 *d*, 3 - 10 *d*, 4 - 2 *d*.

of signal transduction mechanism and transcription factors as it was suggested by Matsumura [2];

2. How continuous should be exposure to TCDD in order to cause animal death? Is it possible to prevent death by interrupting exposures to dioxin?

Investigation of these aspects of the TCDD action would have not only theoretical but also practical importance, in particular, when developing safe working regimes in the chloroorganic synthesis industry.

References

1. Tsyrlov, I. B. *Chlorinated Dioxins: Biological and Medical Aspects,* Novosibirsk, **1990;** p. 210.
2. Matsumura, F. *Biochemical Pharmacology.* **1994,** 48**,** 215-224.
3. Kunzevich, A. D.; Troshkin, N. M.; Baulin S. I.; Golovkov, V. F.; Nedoshivin, Yu. K.; Rembovskiy, V. R. *Dokl. Akad. Nauk.* **1995**, 340, 268-270.
4. Litvak, V. V.; Goryunov, L. I.; Steingarts, V. D. *Zh. Org. Chem*. **1986,** 22, 157-163.
5. Omura, T.; Sato, R. *J. Biol. Chem*. **1964,** 239, 2370-2385.
6. Burke, M. D.; Mayer, R. T. *Drug Metab. Dispos*. **1974,** 2, 583-588.
7. Burke, M. D.; Mayer, R. T.; Koury, R. E. *Cancer Res.* **1977,** 37, 460-463.
8. Smith, A. G.; Clothier, B.; Robinson, S.; Scullion, M. J.; Carthew, P.; Edwards, R.; Luo, J.; Lim, C. K.; Toledano, M. *Mol. Pharmacol*. **1998,** 53, 52-61.
9. Wrighton, S. A.; Campanile, C.; Thomas, P. E. *Mol. Pharmacol*. **1986,** 29, 405-410.
10. Whitlock, J. P. Jr.; Denison, M. S.; Fisher, J. M.; Shen, E. S. *Mol. Biol. Med.* **1989,** 6, 169-178.