The role of flavin enzymes in the pathogenesis of dioxin intoxication.

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The extreme biological activity of dioxins and their structural analogs as well as their wide distribution in the environmental make for the danger of the harmful exposure of population. This is the reason for the sources of technical contamination and the fate of dioxins in the environment.

The risk of pathological development has been estimated both by a single and a chronical effect of the toxin on a human organism and on animals. The after-effects of dioxin exposure are much less studied from the point of view of clinico-structure-biochemical changes. This is partly due to very few registered cases of harmful effects and too late revealing of them. On the other hand, so few number of registered cases of chronic intoxication may be explained by a long latent period of clinic signs as well as by weak specificity of syndromes (not taking into account chloracne), forming during the dioxin intoxication.

Many investigations are devoted to the mechanism of dioxin action. But the investigators attention is concentrated mainly on the possible triggering mechanism of the intoxication - the activation of Cytochrome P-450, and little attention is paid to the intoxication pathogenesis. This is, probably, the reason for so insufficient effect of the remedies, recommended for the treatment and prophylaxis of dioxin intoxication.

The analysis of clinical signs of dioxin pathology in human beings and primates (skin lesions, long-term latent periods, disturbed pigment metabolism) proves that the leading role in the hypothetical theory of the dioxin intoxication pathogenesis belongs to the disturbance of flavin metabolism, in particular, the riboflavin metabolism.

The long-term latent period of the acute intoxication development caused by an extremely toxic agent makes it possible to conclude that the leading role in the pathogenesis is played by the gradually enhancing disturbance in one of the enzymatic systems of an organism, and the activation of Cytochrome P-450 is the triggering mechanism of the process.

The biological cycle of the flavin enzymes as a system in a phylogenetic sense is one of the oldest cycles and consequently has the most firm links with other biological systems, and this is the reason for the appearance of lesions in various systems and organs at later stages of the disease or with the toxin dose increase.

The above described disease signs are characteristic of riboflavin deficiency [1]. At the same time the disturbance of a reproductive function, the embryotoxic and teratogenic effects typical to large doses of TCDD, may be, for example, the result of the

pantothenic acid deficit conjugated with the deficit of riboflavin [2]. The pantothenic acid deficit leads to hydronephrosis, hydrocephaly, lung defects, non-knitted hard palate, microphthalmia of a fetus. These very defects, excluding the last of the listed abnormalities, were registered by the authors in the fetus due to the dioxin intoxication of pregnant rats.

It is considered that one of the main causes of mammals death due to the dioxin intoxication is hypoglycemia as the result of the insulin metabolism disturbance [3], but from our point of view one of the causes of hypoglycemia may be again the mentioned hyporiboflavinosis, accompanied by the deficiency of the active form of pyridocoalphosphate which is a coenzymatic part of glycogenphosphorylase.

The hypothyroxinemia due to the TCDD intoxication may be caused by the disturbance of the porphyrin metabolism manifected by the break of the heme synthesis, which is regulated, by riboflavin [4]. The heme synthesis break leads to the changes in the activity of heme-containing enzyme-iodinase which transforms thyrosin into thyroxin [5,6].

The immunosupression, typical to dioxin intoxication, considering it's early development, is most probably the result of Cytochrome-450 induction. Nevertheless the deficiency of both thyroxin and biotin may be a supporting factor. More over the biotin may be one of the causes of the weight loss up to cachexia.

Basing on these observations the authors formulated a hypothetical scheme for the role of flavin enzymes in the dioxin intoxication pathogenesis. The leading role is applied to riboflavin, which is also structurally similar to TCDD.

The riboflavin ability to interact with Ah-receptors demonstrated in 1985 by Kerl P. Wetal makes it possible to suggest that TCDD in this case plays the role of a coenzyme in the flavin system of metabolism instead of riboflavin, because of the structural similarity .

To check the hypothesis about the pathogenetic role of flavin enzymes on the first stage it seems expedient to carry out an experiment to estimate the efficiency of riboflavin treatment of the dioxin intoxication.

We realize that in case of getting a negative result one can't definitely reject the above mentioned hypothesis because this negative result may be the consequence of abnormal processes of riboflavin biotransformation.

The tests were carried out on white mongrel rats with a mass of 140-200 g (8 animals a group). 2,3,7,8-TCDD was introduced intragastrically in a dose of 1/16 LD_{50} (table 1).

Table 1.

The 2,3,7,8-TCDD acute toxicity parameters for rats after a single intragastric injection (mg/kg).

LD_{50}	LD_{50} + Sx (confidential interval)	LD_{84}
24	50 + 11,4 (26,7 ---------- 73,4)	103

The animals death was delayed depending on the dose till the 19th-54th day. No clear "dose-death date" relation was revealed. The LD_{16} exposure made the animals flaccid, retarded, they refused from food, gathered into groups, were sitting with their paws clasped, shrinking. Most of the rats became dishevelled. Two weeks later the retardation changed into agitation. Some animals demonstrated diarrhea, bloody nose and eyes discharges. The eyes retracted and grew turbid. On the tails and paws bleeding ulcers appeared. The tail often turned blue and later on dried in. During the second month inflammatory skin lesions, together with hair shedding appeared. One could observe both the even hypotrichosis of the whole body and the foci of total baldness (alopecia) on the faces, necks and backs.

The most characteristic and stable sign of the dioxin intoxication is the delayed body mass growth. The body mass was registered on the 1,7,14,21,26,35,42 and 50th days after the TCDD exposure. The true difference in the body mass growth (about 7-10%) after the dioxin exposure of 1/16 LD_{50} is evident since the 14th day till the end of the observation (table 2).

Table 2.

The body mass growth of white rats due to the acute intragastrical 2,3,7,8-TCDD (1/16 LD_{50}) intoxication.

Groups of animals	The body mass growth of the animals (in %%)				
	1 day	7 days	14 days	21 days	26 days
intact	6,2	8,8	20,8	25,9	27,4
dioxin	1.7 [a]	4,5 [a]	0,8 [a]	9,5 [a]	7.4 [a]
dioxin + riboflavin	2,9	7.1 [b]	13,5 [b]	13,5 [a]	15.6 [b]

a - deviations from the control are statistically reliable (P>0,05);
b - deviations from the dioxin group of animals are statistically reliable (P>0.05).

The statistic processing was done by the method of Vilcoxon-Mann-Whitny.

In 26 days after the 2,3,7,8-TCDD exposure blood samples from the sublingual vein was examined to determine the content of glucose, lactate, general lipids, triglycerides, general protein, urea as well as the activity of transaminase.

It is statistically determined that the glucose and alanine-transaminase levels lowered, but the lactate, general lipids and asparagin-transaminase content increased (table 3).

Table 3.
Changes in the biochemical indices of blood in the rats after a single dose of 2,3,7,8-TCDD (1/16 LD_{50}) .

Groups of animals	The body mass growth of the animals (in %%)				
	content of :			transaminase activity	
	glucose	lipids	lactate	AST	ALT
intact	76,24 +3,95	2,27 +0,17	28,38 +2,86	1,68 +0,07	1,16 +0,13
dioxin	62,69 +1,32 [a]	2,89 +0,21 [a]	36,76 +3,27 [a]	2,39 +0,14 [a]	0,83 +0,04 [a]
dioxin + riboflavin	48,86 +3.37 [a,b]	2,75 +0,27 [a]	28,89 +0,95 [b]	2,20 +0,09 [a,b]	0,92 +0,06 [a,b]

a - deviations from the control are statistically reliable (P>0,05);
b - deviations from the dioxin group of animals are statistically reliable (P>0,05).

The statistic processing was done by the method of Fischer - Student.

The therapeutic efficiency of riboflavin was investigated after a single dose (1/16 LD_{50}) of 2,3,7,8-TCDD. The riboflavin was introduced intragastrically once a day in a dose 0,5 mg/kg during 26 days.

On riboflavin introduction after the dioxin intoxication the animals behavior and their body mass growth did not differ from the control group of rats.

Thus the experiment confirmed the therapeutic efficiency of riboflavin against the dioxin intoxication. Later on it seems necessary to clear up the piculiarities of the riboflavin deficiency development. This might help to find the most vulnerable element of the dioxin intoxication pathogenesis and determine the means for it's secondary prophylaxis.

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