ANABOLIC EFFECTS OF THYROXIN IN LIVER OF RATS AFTER EXPERIMENTAL SUBCHRONIC INTOXICATION BY HERBICIDE 2,4-DMA

Edward F. Agletdinov, Alexander Zh. Gilmanov, Guzel M. Abdullina and Felix Kh. Kamilov

Chair of Biochemistry, Bashkir State Medical University, Ufa, 450000, Russia.

Introduction

The experimental intoxication by 2,4-D disrupt the serum levels of several enzymes and components which mainly reflect damages of liver and other organs related to herbicide[1,2]. We previously reported on changes of serum thyroid hormonal spectrum and enzyme activity in liver homogenate of rats at experimental 2,4-D dimethylamine salt (2,4-DMA) action that may possibly be connected to hormonal disturbances[3,4,5]. However the detailed mechanism of chlorinated aromatic compounds' damage of pituitary-thyroid-target tissue axis seem to be not sufficiently clear. The aim of this investigation was to determine possible changes of protein synthesis and content (as thyroid-dependent processes) in the liver of rats in conditions of subchronic 4-week intoxication by 2,4-DMA and probable influence of exogenous thyroxin on these parameters, as serum T4 level under action of the toxicant was shown to be generally decreased[3,4].

Materials and Methods

The experiments were performed on 66 male rats of 180-230 grams body weight. For modeling of subchronic intoxication rats received daily the 2,4-DMA water solution intragastrally during 4 weeks in total doses equivalent to LD_{50} and 1/10 LD_{50}. Control animals received sodium chloride solution. Upon the termination of the experiment the rats were decapitated, livers homogenated, cytosol and mitochondrial fractions separated by centrifugation. The protein content in fractions was measured using the Lowry method, and the results were expressed relatively to native tissue weight.

For evaluation of protein synthesis in liver, the rats after intoxication were injected intraperitoneally with ^{14}C-labeled protein hydrolisate (Reanal, Hungary; 1 mCi/kg body weight). Two hours later the animals were decapitated, a pieces of liver (~0.5 g) collected, and the liver proteins were separated from other tissue components by multiple extraction. The amount of incorporated ^{14}C by beta-activity of protein samples was measured and expressed relatively to general protein content. Separate groups of control and intoxicated animals at 48 hours before decapitation were unitary injected intraperitoneally with the solution of L-thyroxin (Sigma, USA; 1 mg/100 g body weight). The protein content and intensity of its synthesis in those animals were estimated the above mentioned way. All statistic data processing was performed using Student t-criterion.

Results and Discussion

The subchronic 28-day intoxication by LD_{50} of 2,4-DMA in rats was accompanied with 13.8% decrease in mitochondrial protein content, whereas in cytosol fraction it was contralaterally increased up to 8,4% (Table 1). This result can testify about the slight inductive abilities of 2,4-DMA at intoxication, probably connected to induction of microsomal oxidation enzyme synthesis. At the same time a relative decrease in mitochondrial protein content in hepatocytes observed in

our investigations. As the oppression of some enzymes mainly located at mitochondria we had found, it can generally testify about some delay of amino acid catabolism and probable disorders of specific protein transport into mitochondria in hepatocytes at intoxication. The disorders of electrolytes and/or water cell status may also occur.

Table 1. Protein content (mg/g of native tissue) in liver homogenate of rats after 4-week intoxication by 2,4-DMA and after administration of thyroxin (M±m; n=16).

Cell compartment	Control group	Control + thyroxin	LD_{50} of 2,4-DMA	LD_{50} + thyroxin
Mitochondrial protein content	10,63±0,70	10,20±0,35	9,16±0,37*	10,24±0,72
% of control level	---	96,0	86,2	96,3
Cytosol protein content	58,00±1,56	68,00±1,87*	62,85±1,47*	68,92±2,01*
% of control level	---	117,2	108,4	118,8

*- statistically significant changes ($p<0.05$)

The effect of thyroxin in 28-day-intoxicated rats resulted both in cytosol and mitochondria in an elevation in protein content to normal and overnormal values. At the same time the thyroxin action in the control group of animals showed a slight decrease of mitochondrial protein and significant increase in cytosol protein content. Similar values of protein in liver cytosol of rats under the thyroxin effect was found both at intoxication and in the control group. In view of thyroxin-induced relative gain of cytosol protein content in conditions of 2,4-DMA toxic action was smaller compared to the control group, this fact can testify to some "exhaustion" of thyroid-dependent protein synthesis mechanisms, or disorders of periferic deiodination of thyroxin in liver of rats under intoxication. Anyway, the direct investigation of protein synthesis in liver is of great interest.

Our experiments at 4-week 2,4-DMA intoxication in rats showed the reduction of ^{14}C-labeled amino acids incorporation into the common liver proteins. The changes observed were dose-dependent: from slight (5.3%) at 1/10 LD_{50} to significant (31.4%) at LD_{50} of toxicant (Table 2). We also found the reduction of ^{14}C incorporation into the liver proteins in control animals as the effect of thyroxin. Taking into account the same influence of this hormone on mitochondrial protein content, it could be explained with general disorders of anabolism in euthyroid rats under exogenous thyroxin action, but the significant increase in cytosol protein content mentioned above doesn't meet these facts. The reduction of catabolic reactions at intoxication may also occur.

Table 2. Intensity of ^{14}C incorporation into the liver proteins in rats who received ^{14}C-protein hydrolisate after intoxication by the different doses of 2,4-DMA and after administration of thyroxin (beta-activity of separated liver proteins; pulses per min/mg of protein; M±m)

Group of Animals	Control group	Control + thyroxin	1/10 LD_{50} 2,4-DMA	1/10 LD_{50} + thyroxin	LD_{50} 2,4-DMA	LD_{50} + thyroxin
Activity of liver proteins	76.1±3.8	61.6±3.7*	72.1±2.4	104.4±7.7*	52.3±6.3*	92.4±4.5*
% of control	---	80.8	94.7	137.1	68.6	121.3

*- statistically significant changes ($p<0.05$)

The effect of exogenous thyroxin in intoxicated rats resulted in a significant elevation in ^{14}C amino acids incorporation into the liver proteins to overnormal values. This effect was dose-dependent: the relative increase was 45.7% at 1/10 LD_{50} and 76.8% at LD_{50} doses of 2,4-DMA. This generally testify about the safety of thyroid-dependent inductive abilities of cell anabolic systems, and at the same time more correspond to data on relative hypothyroid status occuring in conditions of intoxication. The thyroxin effect is of interest for better understanding of the toxicity mechanisms, and with dose specification it should be taken into account in the development of metabolic correction at intoxication by phenoxyherbicides and probably other chlorinated aromatic compounds.

References

1. Paulino C., Guerra J., Oliveira G., Palermo Neto J. (1996) Vet Hum Toxicol. *38(5),* 348-52 .
2. Palmeira C., Moreno A., Madeira V. (1994) Arch Toxicol. *68(1)*, 24-31.
3. Kamilov F., Davletov E., Gilmanov A., Galimov Sh. (1997) Organohal Comp. *34*, 178-181.
4. Galimov Sh., Gilmanov A., Kamilov F., Davletov E., Shchepansky V. (1997) Med Tr Prom Ecol. *8*, 15-18.
5. Gilmanov A., Abdullina G., Kamilov F. (1999) Organohal Comp. *42*, 317-320.