EXOGENOUS THYROXIN PARTIALLY CORRECTS THE CHANGES OF ADENINE NUCLEOTIDES CONTENT IN LIVER OF RATS UNDER THE ACTION OF HERBICIDE 2,4-DMA

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Introduction

It is now established that energy deficiency is one of the main reasons of cell injury and death in any intoxication. This can be either caused by elevated energy utilization in detoxication reactions, or by insufficient ATP synthesis as result of the damage of mitochondrial oxidative phosphorilation chain[1,2]. The toxicity of phenoxy herbicides bases in particular on its intervention into cell energy metabolism leading to energy deficiency and general metabolic disorders, first of all in hepatocytes[2,3]. One of the early markers of cell energy disorders is the change of adenine nucleotide content and correlation.

It is well known that the synthesis and activity of the most cell enzymes is the subject of thyroid hormonal influence. We've previously reported on changes of serum thyroid hormonal spectrum, enzyme activity and oxydative metabolism intensity in liver cells of rats at experimental 2,4-DMA action[4,5,6]. Our interest was to study the changes of adenine nucleotide content and correlation in liver homogenate of rats after 4-week intoxication by 2,4-DMA and probable influence of exogenous thyroxin on these parameters, as serum T4 level under the action of toxicant was shown to be generally decreased[4].

Methods and Materials

The experiments were performed on 86 male rats of 150-210 grams body weight. The animals were given the water solution of 2,4-DMA intragastrally every day within four weeks, the total dose of toxicant was equivalent to LD_{50} and 1/10 LD_{50} for all period. The control animals were given the same quantity of 0.9% sodium chloride solution. Upon the termination of experiment all rats were decapitated, the pieces of liver were taken and frozen in liquid nitrogen. Adenine nucleotides were extracted from the tissue powder with 6% $HClO_4$, and enzymatic analysis of supernatant was performed immediately. The ATP content was measured by the method of Lamprecht and Trautschold using hexokinase and glucose-6-phosphate dehydrogenase, ADP - by Javorek and Gruber using PEP, pyruvate kinase and LDH[7]. For determination of AMP the same method was used after conversion of AMP to ADP by addition plenty of ATP and myokinase. The total adenine nucleotide content and ATP/ADP ratio were also calculated in each case.

For evaluation of exogenous thyroxin action on adenine nucleotide content the separate intoxicated animals were additionally injected intraperitoneally with L-thyroxin solution ("Sigma", 1 mg/100 g body weight) 48 hours before decapitation. The control animals were received 0.9% sodium chloride.

Statistical reliability of the results was performed by variance analysis and Student's t-test.

Results and Discussion

The results of our study presented in Table 1 show the considerable dose-dependent shifts of adenine nucleotides content in liver tissue of rats as response to the action of 2,4-DMA. There was a significant decrease in the ATP level by 23.9% at 1/10 LD_{50} toxicant total dose and by 48.2% at LD_{50} total dose. In parallel the ADP and AMP level was elevated to 169.3% and 199.4% of the control values, respectively. The ATP/ADP ratio under the action of 2,4-DMA was strongly decreased on 32.6 – 65.1%, whereas the total adenine nucleotide content was only slightly changed.

Similar shifts were observed under the action of phenoxy herbicides on rat hepatocytes in vitro: the decrease of ATP and parallel increase of ADP and AMP level testify about the prevalence of ATP degradation over its synthesis[1,3]. The authors consider the liver pathology at the early stage of 2,4-D intoxication connected with insufficient cell energy supply. The shifts found out in our experiments may also be the consequence of the retardation of ATP synthesis and/or acceleration of its degradation. At the same time the slight changes of total adenine nucleotide content reflect only a little influence of the toxicant on general nucleotide synthesis and/or degradation. Taking into consideration the reduction of energy oxidative metabolism in liver tissue under the action of 2,4-DMA[3,6] we can suppose that the main reason of the shifts obtained in our experiments is the slowing of ATP synthesis caused probably by alteration of mitochondrial membranes[6] and its enzymes by the herbicide itself and its metabolites as well as by the products of activated peroxidation. The development of different metabolic blockages and hyper-accumulation of intermediate metabolites may also occur[1].

Table 1: Adenine Nucleotide Content in Liver Tissue of Rats at 28-day Intoxication by 2,4-DMA (M±m; n=12)

Group of animals	**Control**	**1/10 LD_{50} 2,4-DMA**	**LD_{50} 2,4-DMA**
ATP, umol/g of tissue % of the control value	237.0 ± 11.2 ---	180.3 ± 11.6 76.1*	133.0 ± 12.5 59.8*
ADP, umol/g of tissue % of the control value	88.0 ± 6.1 ---	101.0 ± 6.6 114.8	149.0 ± 8.7 169.3*
AMP, umol/g of tissue % of the control value	17.3 ± 2.9 ---	22.8 ± 2.9 131.9	34.5 ± 3.6 199.4*
ATP+ADP+AMP, umol/g of tissue % of the control value	345.0 ± 22.5 ---	304.0 ± 21.1 88.1	335.3 ± 22.6 97.1
ATP/ADP % of the control value	2.64 ± 0.08 ---	1.78 ± 0.11 67.4*	0.92 ± 0.12 34.9*

*- statistically significant ($p<0.05$)

The consequence of nucleotide content changes is the critical fall of ATP/ADP ratio down to 34.9% of the control value. These shifts of the index should stimulate cell energy metabolism, but the decrease of enzyme activity observed in our early investigations[5] may testify about disorders of enzyme synthesis de novo caused by relative intracellular hypothyroid status.

The direct influence of thyroid hormones on energy metabolism is most well-known by its different metabolic effects. In our experiments the use of exogenous T4 in intoxicated rats led to 37.2% elevation of ATP content in liver tissue and its approach to the control level, but remaining

significatly lower (see Table 2). Contralateral shifts were observed in ADP and AMP content; its levels remained some higher of the control value. This resulted in about 2 times elevation of the ATP/ADP ratio and its noticeable shift to the control level. Taking into view that T4 administration did not affect the total nucleotide content in intoxicated rats but significantly activated energy oxidative metabolism[6], our results directly testify about the acceleration of ATP synthesis in liver tissue. At the same time the remaining residual shifts of adenine nucleotide level even under T4 influence show that the restoration of intracellular thyroid status is important but not the only factor of energy metabolism normalization at intoxication by 2,4-DMA.

Table 2: Adenine Nucleotide Content in Liver Tissue of Rats at 28-day Intoxication by 2,4-DMA and under the Influence of Exogenous Thyroxin (M±m; n=12).

Group of animals	**Control**	**LD_{50} 2,4-DMA**	**LD_{50} 2,4-DMA + + Thyroxin**
ATP, umol/g of tissue	237.0 ± 11.2	141.8 ± 10.3	194,5 ± 9,2
% of the control value	---	59.8*	82.1*
% of the 2,4-DMA group value	---	---	137.2**
ADP, umol/g of tissue	88.0 ± 6.1	149.0 ± 8.7	103.5 ± 4.6
% of the control value	---	169.3*	117.6
% of the 2,4-DMA group value		---	69.7**
AMP, umol/g of tissue	17.3 ± 2.9	34.5 ± 3.6	27.0 ± 4.2
% of the control value	---	199.4*	156.5*
% of the 2,4-DMA group value	---	---	78.3
ATP+ADP+AMP, umol/g of tissue	345.0 ± 22.5	335.3 ± 22.6	325.0 ± 17.9
% of the control value	---	97.1	94.2
% of the 2,4-DMA group value	---	---	96.9
ATP/ADP	2.64 ± 0.08	0.92 ± 0.12	1.88 ± 0.06
% of the control value	---	34.9*	69.9*
% of the 2,4-DMA group value	---	---	197.9**

*- statistically significant ($p<0.05$) by the control value

** *- statistically significant ($p<0.05$) by the 2,4-DMA group value

Meaning the role of ATP in metabolism we suppose that the significant part of metabolic disorders developing at phenoxy herbicide intoxication may be directly or indirectly connected with cellular ATP insufficiency. This accompanies with the limitation of energy-depended cell reactions, including vitally significant ones.

References

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