An impact of compounds of imidazoline on speed of oxygen intake by mitochondria of rats' hepatocyte

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Abstract

A main problem of modern medicine is revelation of biochemical mechanisms of pathological processes development during impact on xenobiotics organism (XO). Compounds of imidazoline are compounds of xenobiotics organism components of which according to physical and chemical peculiarities and molecules structure properties contain group of cationic surface active substances. Compounds of imidazoline are characterized by significant synthesis volume, wide use in different fields of economic and intake in water sources and possible impact on population health.

Previous investigations determined long-lasting rats' toxification by compounds of imidazoline with alkyl radicals S7-9 and S9-15 in doses 1/10 and 1/100 DL50 initiates the development of oxidative stress with the destruction of protein and lipid molecule.

Aim of the work was to study the speed of oxygen intake by mitochondria of rats' hepatocytes on the 30th day of compounds of imidazoline impact in dose 1/100 DL 50.

Keywords: xenobiotics, imidazolines , mitochondria , hepatocyte .

1. Introduction

One of the main problems of modern medicine is revelation of biochemical mechanisms of pathological processes development during impact on xenobiotics organism (XO) [1, 2]. Compounds of imidazoline are compounds of xenobiotics organism components of which according to physical and chemical peculiarities and molecules structure properties contain group of cationic surface active substances. Compounds of imidazoline are characterized by significant synthesis volume, wide use in different fields of economics (such as basis of industrial output of household agents, antistatic substances, anticorrosive drugs, adhesive supplements and others), and also intake in water sources and possible impact on population health [3-5].

Previous investigations determined long-lasting rats' toxification by compounds of imidazoline with alkyl radicals S7-9 and S9-15 in doses 1/10 and 1/100 DL50 initiates the development of oxidative stress with the destruction of protein and lipid molecules, that is approved by the increase an intensity of chemicoluminescence in organs and tissues, which contains products of thiobarbituric acid, carbonyl groups of oxidative modified proteins [6]. In such way products of oxidative modification of proteins and lipid peroxidation as consequence of high reactive activity and selectivity in biological action can be as the main key, which limits the condition of resistance of organism to long-lasting impact of compounds of imidazoline through change of physical and chemical peculiarities of biomembranes, activity of membranous enzymatic and receptor complexes. Free-radiated acidification, which intensifies during long-lasting impact of compounds of imidazoline can be one of the most important mechanisms of damage of membranous respiratory chain of mitochondria of hepatocytes.

The aim of the work was to study the speed of oxygen intake by mitochondria of rats' hepatocytes on the 30^{th} day of compounds of imidazoline impact in dose 1/100 DL 50.

2. Materials and methods of research.

Samples of compounds of imidazoline with alkyl radicals S7-9 (compounds of imidazoline7-9) and S9-15 (compounds of imidazoline9-15) were used in this work. Experiments were done on sexually mature rats of WAG, with body weight (180-220) gram. Manipulations with animals were done according to the main principles of bioethics. They were exposed to peroral coarse with probe by water solution of compounds daily and once time during 30 days in such doses as 1/10 and 1/100 DL50. Doses of medial lethal time (DL50) included: for compounds of imidazoline 7-9 - 1,8 g/kg; compounds of imidazoline 9-15 - 5,0 g/kg of body's weight. Animals of control were taken volumes of fresh water. Investigation of indices was done in 30 days after experiment's start. Stall was done by decapitation; previously it was taken anesthesia by thiopental sodium.

To get rat liver homogenate part of tissue was cut on cold, homogenized with glass Potter homogenizer with teflon pestle in freeze environment displacement (0,25 M of sucrose solution on 0,01 M Tris-HCI buffer, pH-7,4 with supplement 1 mm of EDTA), correlation tissue/environment (weight/volume) contained 1 g/9ml. Secretion of subcellular fractions of rats' liver was done by differential centrifugation. During the first stage 10% filtered homogenate was centrifuged on centrifuge at 600 g. during 10 minutes in order to separate undamaged cellular elements and nuclei. During the second stage mitochondrial fraction was defined. Nuclear free fraction of liver's homogenate was centrifuged at 8500 g during 10 min., deposit was washed twice 0,25 M by sucrose in 0,01 M Tris-HCI buffer (pH 7,4) and centrifuged at 8500 g during 10 min. Suspension of mitochondria was done by resuspending of deposit of washed organelles in secretion environment. Speed of oxygen intake by mitochondria of rats' hepatocyte was estimated by polarographic method [7, 8], adding specific substrates and inhibitors of the first and the second complexes of respiratory chain. Next parameters were used: 1) condition V_4 – is high content of substrates of the first complex in

incubation - 5 mm of glutamate and 5 mm of malate or substrate of the second complex – succinate without ADP; 2) condition V_3 is similar to V_4, but in the presence of 200 microns of ADP (respiratory chain is a factor which limits reaction speed) 3) condition V_d – presents the same conditions that in condition V_4, but in presence an uncoupler of peroxidation and phosphorylation of 30 microns of 2,4 dinitrophenol.

Statistic analysis was done to process statistic information Statistica 6.1 (StatSoft, Inc., USA). Parametric peculiarities were used such as medial index (M) and medial quadric defection (deviation) (s); in the case of its absence nonparametric ones – median (Me) and interquartile amplitude. For critical level of importance at control $p<0,05$ was taken during statistic hypothesis screening.

3. Study's results

On the 30th day of compounds of imidazoline action ($p\leq0,015$) was observed, according to control group increase of respiration speed of mitochondria by rats' hepatocytes in conditions V_3 and V_4 on substrates of the first complex (nicotinamide adenine dinucleotide (NAD) coenzyme *Q*-oxidoreductase) at average on 41 and 28% correspondingly. It was defined based on this ground statistically accurate decrease ($p\leq0,001$) of respiration speed in V_3 on substrates of the 2nd complex (succinate-coenzyme *Q*-oxidoreductase) of respiratory chain complex: on 45% in compounds of imidazoline 7-9 and on 20% - in compounds of imidazoline 9-15. Intensity of mitochondria's respiration on succinate as it was in condition V_4, statistically decreased only by compounds of imidazoline 7-9 (on 35%). For long-lasting action of compounds of imidazoline 9-15 it was also determined a tendency to decrease, but it was not accurate ($p=0,165$). Addition of 2,4 dinitrophenol caused decrease ($p<0,001$), as related to control, speed of respiration of rats' mitochondria in substrates of the 2nd complex of respiratory chain: on 38% for compounds of imidazoline7-9 and only on 18% for compounds of imidazoline9-15. It should be noted intensity of oxygen intake by mitochondria of rats' hepatocytes in V_d on glutamate and malate was not practically changed ($p=0,085$ and $p=0,836$).

Received results demonstrate long-lasting impact of compounds of imidazoline causes malformation of mitochondrial respiratory chain of rats' hepatocytes, especially in the work of the 2nd complex (succinate-coenzyme Q- oxidoreductase). Detected changes in the first complex work possible can be compensator character. But on the other hand it is known this complex can realize one-electron repair of oxygen in superoxide-anion. In hepatocytes of normal liver intensity of this process is on low level thanks to antioxidant protection in mitochondria, that's why it doesn't cause impact on respiratory chain. It can be supposed the increase of the 1st complex work of mitochondrial respiratory chain of rats' hepatocytes in long-lasting impact of compounds of imidazoline is connected with damage of electron's transport that can cause number of superoxide-anion. Results of oxidation and phosphorylation processes define separation on substrates of the 2nd complex of respiratory chain.

4. Conclusions

So, long-lasting intoxication of rats' organism by compounds of imidazoline causes structural and functional damages of mitochondrial respiratory chain of rats' hepatocytes that is approved by speed increase of mitochondria respiration in condition V_3 and V_4 on substrates of the 1st complex as related to speed decrease of mitochondria respiration in condition V_3 and V_4 on substrates of the 2nd complex, separation of acidification and phosphorylation on substrates of the 2nd complex.

Detected damages should be defined as one of the pathogenetic action of membranous mechanism of compounds of imidazoline action.

Table

Speed of oxygen intake by mitochondria of rats' hepatocytes on the 30th day after imidazoline action in dose 1/100 (DL 50)

(nM O_2/min mg of protein, n=15; Me [25%; 75%] or M±s)

Index	Control	CI7-9	CI9-15
Condition 3 (V_3) on succinate	50,2 [44,4; 52,3]	27,6±8,80 p<0,001	40,1 [36,2; 40,4] p=0,001
Condition 3 (V_3) on succinate and malate	29,8 [25,0; 30,5]	44,4±4,32 p<0,001	39,3±5,45 p<0,001
Condition 4 (V_4) on succinate	12,6±3,64	8,17±1,71 p=0,001	10,8±2,62 p=0,165
Condition 4 (V_4) on glutamate and malate	5,40±1,26	7,8 [6,8; 10,7] p<0,001	6,63±1,29 p=0,015
Condition of 2,4- dinitrophenol (V_d) on succinate	53,6±6,21	33,3 [27,0; 38,5] p<0,001	43,8±4,66 p<0,001
Condition of 2,4- dinitrophenol (V_d) on glutamate and malate	24,4±6,04	19,4 [17,7; 22,2] p=0,085	25,6±7,16 p=0,836

Note: p is the level of importance as related to control

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