An impact of oligoesters of polyatomic alcohol on the content of cytochromes in microsomal fraction of rats' liver

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Abstract

Study of mechanisms of pathological processes development is actual one in modern science. Impact of xenobiotics on organism is also important and actual study in modern science. Defined ways of organism adaptation to xenobiotics action were formed during evolution, central role in which presents biochemical mechanisms of their decontamination.

Samples of OEF-LP 502 (polyoxypropylene glycol) and 503 (polyoxypropylene triol) were used in this work with defined physical and chemical peculiarities. Experiments were done on sexually mature rats of Wistar with body weight (180-220) gram.

Long-lasting impact of OEF-LP 502 and 503 in doses 1/10 and 1/100 LD50 is characterized by destruction of functional condition of rats' liver microsomes that is defined by decrease of cytochrome P450 and b5 content. Increase of cytochrome level on the 15th day of substances impact is adaptive reaction of organism to xenobiotics intake, which is connected with decontamination process, but on the other hand with generation of active forms of oxygen and products of lipid peroxidation.

Keywords: oligoesters of polyatomic alcohol, cytochromes, microsomal fraction, xenobiotics.

1. Introduction

Study of mechanisms of pathological processes development is actual one in modern science. Impact of xenobiotics on organism is also important and actual study in modern science [4,9]. Oligoesters of polyatomic alcohol «Laprol» Polypropylene glycol (OEF-LP) present wide spread xenobiotics, which are characterized by significant synthesis volume, wide use (as basis of industrial output of synthetic resin, foam polyurethane, paint materials, household agents), intake in water sources of population and as a result impact on person's health [2]. Defined ways of organism adaptation to xenobiotics action were formed during evolution, central role in which presents biochemical mechanisms of their decontamination [8,11,12,18]. Last as a rule are connected with functioning monooxygenase system (MS) of smooth endoplasmic reticulum.

Main peculiarities of monooxygenase system present: ability to induction in actions of many exogenic and endogenic combinations [14, 17], organ and tissue peculiarity, specific and individual variability [10, 15]. In microsomal membrane of hepatocytes two enzymatic systems are localized, acidifying $NADPH_2$ and $NADH_2$. NADPH is dependent system which acts with cytochrome P450 as terminal element, but NADH is system with cytochrome b5 as acceptor of electrons. Cytochrome b5 can participate in hydroxylation processes, be a donor of electrons for cytochrome P450 [2]. Besides monooxygenase system is a main source in order to form new active oxygen forms [16, 22].

Enzymatic and nonenzymatic systems of lipid peroxidation are present in microsomal membranes [3]. It was determined monooxygenase system activation can cause the development of oxidative stress [13, 20]. Hepatocytes damage in monooxygenase system is one of the main reasons of homeostasis imbalance, pathological processes development. This question during long-lasting impact of new representatives of «Laprol» has not studied yet, so the information about its condition is necessary for detailed mechanism study of biological action and also development of ways of their correction.

The aim of the investigation is to evaluate the impact of OEF-LP 502 and 503 in dynamics in doses 1/10 and 1/100 LD50 on cytochromes P450 and b5 content in microsomal fraction of rats' liver.

2. Materials and methods of research

Samples of OEF-LP 502 (polyoxypropylene glycol) and 503 (polyoxypropylene triol) were used in this work with defined physical and chemical peculiarities. Experiments were done on sexually mature rats of Wistar 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 45 days in doses 1/10 and 1/100 LD50. Doses of medial lethal time (DL50) included for OEF-LP-502 - 1,83 g/kg; OEF-LP-503 - 21,3 g/kg of body's weight. Animals of control group were taken volumes of fresh water. Investigation of indices was done in dynamics on the 15^{th}, 30^{th}, 45^{th} days after experiment's start. Each group contained 10 animals. Rats were decapitated, previously it was taken anesthesia by thiopental sodium in 50 mg/kg of body's weight. Secretion of subcellular fraction of rats' liver was done by differential centrifugation. 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. Cytochromes content of P450 and b5 suspension of microsomes of rats' liver was detected by spectrophotometrically on biradiate spectrophotometer «Specord UV VIS» [6].

It was considered inequality in absorption of oxidative and restorative form of hemoproteins to determine cytochrome b5. It was measured volume of absorption of complex of restorative cytochrome

P450 with carbonous oxide at 450 nm to determine cytochrome P450. Comparison of average number in selections with normal division was done due t-criterion (Student's test) statistic criterion $p<0,05$ was equal to critical level.

3. Study's results

On the 15th day of experiment it was detrained that OEF-LP-502 in doses 1/10 and 1/100 LD50 causes microsomal fraction of rats' liver to statistically important ($p\leq0,015$), compared with control group of animals, increase of cytochrome P450 on 110 and 37% correspondingly (table). So, it was observed increase ($p\leq0,016$) of cytochrome b5, correspondingly on 70 and 30%. For OEF-LP-503 on 15 days in dose 1/10 LD50 was characterized by accurate ($p\leq0,013$), compared with control increase of cytochrome P450 and b5 (in average on 63 and 14% correspondingly), when in dose 1/100 LD50 changes were not registered ($p>0,05$).

On the 30th day of experiment it was observed decrease of microsomal cytochromes in rats' liver. So, OEF-LP-502 and OEF-LP-503 in dose 1/10 LD50, in this time it was ($p<0,001$), compared with control group of animals and also decrease of cytochrome P450 correspondingly on 66 and 49%, and also in dose 1/100 LD50 – on 49 and 41%. Similar tendency was observed in cytochrome b5: decrease in average on 52 and 35% in conditions of impact of OEF-LP- 502 and OEF-LP- 503 in doses 1/10 LD50, on 28 and 14% in dose 1/100 LD 50.

On the 45th day of experiment for cytochromes in microsomal fraction of rats' liver considerable decrease was detected. In dose 1/10 LD50 OEF-LP-502 and OEF-LP-503 it was decreased ($p<0,001$) cytochrome content P450 correspondingly on 73 and 61%, and cytochrome b5 – on 69 and 47%. Cytochrome P450 during long-lasting action of OEF-LP-502 in dose 1/100 LD50 accurately decreased on 58%, and cytochrome b5 – on 42%; as for OEF-LP-503 it contained 55 and 25%.

Cytochrome P450 decrease on the 30th and 45th days of action of investigated substances in doses 1/10 and 1/100 LD50 indicates inhibition of microsomal oxygenase and decrease of biotransformation processes. Such dynamics can be determined by speed dominance of degradation processes of moleclules of cytochrome P450 in result of reactions activation of lipid peroxidation in microsomes of rats' liver [7]. It should be noted results of previous investigations determined [1] on the 30th and 45th days of OEF-LP- 502 and OEF-LP-503 in doses 1/10 and 1/100 LD50 decrease occurs of NADPH-dependent reductased microsomes' activity. This fact is possible determined by decrease of homoprotein cytochrome P450 content, which is terminal part of NADPH-specific electron-transport chain. Decrease of cytochrome b5 on the 30th and the 45th days of action in doses 1/10 and 1/100 LD50 can be connected with destruction of functioning electron-transport chain with its participation. It should be noted on the 30th day of experiment there is increase of NADH-dependent reductase activity [1] that can be a reason of destruction of electrons delivery, in particular not on decreased level of cytochrome b5 but on oxygen with formation of its active forms and subsequent intensification of free-radical reactions in the cell [5]. Cytochrome b5 is a component which passes electrons on cytochromes P450 in microsomal NADH-dependent electron-transport chain [19, 21]. Decrease of its content with subsequent degradation of molecule is connected with intensification of lipid peroxidation processes in membranes of endoplasmic reticulum of hepatocytes as a result of xenobiotics action. There is protection of molecule of terminal oxygenase cytochrome P450, which is also main acceptor of electrons that passes from cytochrome b5; in this case electrons' transition on cytochrome P450 can achieve from NADPH-cytochrome P450 reductase that prevents destruction of cytochrome P450 functioning dependent chain of hydroxylated monooxygenase system.

Adaptive reaction is defined increase of cytochromes content of microsomes of rats' liver on the 15th days of observation during substances' action in doses 1/10 and 1/100 LD50. But monooxygenase system

activation in rats' liver assists in detoxification increase of examined substances and also activation of free-radical processes. It can be supposed OEF can be oxidated in microsomes of rats' liver free radicals which initiates processes of lipid peroxidation with subsequent destruction of membranous structure of hepatocytes, and also destruction of functional activity of enzymatic systems of xenobiotics decontamination [21].

4. Conclusions

Long-lasting impact of OEF-LP 502 and 503 in doses 1/10 and 1/100 LD50 is characterized by destruction of functional condition of rats' liver microsomes that is defined by decrease of cytochrome P450 and b5 content. Increase of cytochrome level on the 15th day of substances impact is adaptive reaction of organism to xenobiotics intake, which is connected with decontamination process, but on the other hand with generation of active forms of oxygen and products of lipid peroxidation.

Table

Cytochrome's content in microsomal fraction of rats' liver during OEF-502 and OEF-503 M±m, n=10)

Substance	Cytochrome P450, (nM) nanometers /mg of protein			Cytochrome b5, (nM) nanometers/mg of protein		
	Day of experiment (observation)					
	15	30	45	15	30	45
Dose 1/10 LD50						
OEF-LP -502	1,89± 0,076 p<0,001	0,31± 0,017 p<0,001	0,25± 0,016 p<0,001	0,97± 0,032 p<0,001	0,31± 0,021 p<0,001	0,17± 0,011 p<0,001
OEF-LP -503	1,47± 0,064 p<0,001	0,47± 0,016 p<0,001	0,36± 0,019 p<0,001	0,65± 0,026 p=0,013	0,42± 0,023 p<0,001	0,29± 0,022 p<0,001
Dose 1/100 LD50						
OEF-LP -502	1,23± 0,105 p=0,015	0,47± 0,021 p<0,001	0,39± 0,011 p<0,001	0,74± 0,062 p=0,016	0,47± 0,039 p=0,001	0,32± 0,027 p<0,001
OEF-LP -503	0,92± 0,055 p=0,844	0,54± 0,035 p<0,001	0,42± 0,032 p<0,001	0,62± 0,006 p=0,055	0,56± 0,021 p=0,005	0,41± 0,014 p<0,001
Control	0,90± 0,065	0,92± 0,051	0,93± 0,044	0,57± 0,006	0,65± 0,018	0,55± 0,015

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

5. Literature

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