БИОЛОГИЯ

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ACUTE TOXIC HEPATOPATHY IN THE EXPERIMENT AND ITS CORRECTION BY HEPATOPROTECTORS

Abstract. One One of frequent complications of acute exogenous poisonings is the toxic hepatopathy. Entering of toxic substances into an organism and their subsequent metabolization result in increased formation of free radicals. The identification of the HOL role when acute poisoning leads to the search of new effective drugs with antioxidatic properties. The purpose of our researches is studying of antioxidant properties "Essentiale" and new pharmacological substance amidophosphanata citizin (AFC). All experimental procedures were carried out according to the requirements of GLP (Good Laboratory Practice-appropriate laboratory practice). Acute toxic hepatitis was caused by intraperitoneal introduction of 40% oil CCL_4 solution, in the dose of 0,2 ml/100,0 once. By results of the researches acute poisoning with four-chloride Carbonium resulted in toxic hepatitis. The introduction of the tested pharmacological compound amidophosphanata citizin reduced the increased activity of AlaT, AsaT, amount of blood total bilirubin (TB), as well as the growth of DC (level the diene conjugates in the experienced group 2,7 times lower than in the group of comparison, and 3,8 times more than in the control one) and limited accumulation of SB. Introduction of paracetamol in the increased dose causes toxic hepatitis of the tested animals.

Keywords: Amidophosphanat citizina, hepatoprotector, "Essentiale", hyperoxide oxidation of lipids, four-chloride Carbonium, hepatopathy

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Аннотация. Одним из частых осложнений острых экзогенных отравлений является токсическая гепатопатия. Поступление токсических веществ в организм и их последующая метаболизация приводят к повышенному образованию свободных радикалов. Выявление роли ПОЛ при острых отравлениях приводит к поиску новых эффективных препаратов с антиоксидантными свойствами. Цель исследований - сравнительное изучение анитиоксидантных свойств эссенциале и нового фармакологического вещества амидофосфаната цитизина. Все экспериментальные процедуры были проведены в соответствии с требованиями НЛП (надлежащая лабораторная практика). Острый токсический гепатит вызывали внутрибрюшинным введением 40%

масляного раствора CCl_4, в дозе 0,2 мл/100,0 однократно. По результатам исследований острое отравление четыреххлористым углеродом привело к развитию токсического гепатита. Введение испытуемого фармакологического соединения амидофосфаната цитизина активно и отчетливо происходило сдерживая рост активности АЛТ, АСТ, количества ОБ в крови, а также сдерживая рост ДК (уровень диеновых конъюгат в опытной группе ниже в 2,7 раза, чем в группе сравнения, и в 3,8 раза, чем в контрольной) и ограничивая накопление ШО.

Ключевые слова: Амидофосфанат цитизина, гепатопротектор «Эссенциале», перекисное окисление липидов, четырёххлористый углерод, гепатопатия.

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Түйіндеме. Асқынған экзогенді уланудың жиі тараған асқынуларының бірі – улы гепатопатия. Ағзаға улы заттардың түсуі және олардың соңғы метаболизмі бос радикалдардың көп пайда болуына әкеледі. Қатты улану кездерінде ЛП-ның орнын табу антиоксидантты қасиеттері бар жаңа тиімді заттарды іздеуге әкеледі. Біздің зерттеудің мақсаты – эссенциале мен жаңа фармакологиялық зат – цитизинның амидофосфонатының антиоксидантты қасиеттерін салыстырмалы түрде зерттеу. Барлық эксперименттер ДЗТ- ның (дұрыс зертханалық тәжірибе) талаптары бойынша жүргізілді. Жедел улы гепатитті CCl_4 – тің 40% майлы ерітіндісін 0,2 мл/100,0 мөлшерде бір рет құрсақ қуысына енгізумен тудырдық. Зерттеу нәтижесі бойынша енгізген CCl_4 – улы гепатитке әкелді. Зерттеудегі фармакологиялық зат - цитизинның амидофосфонаты белсенді және нақты қандағы АЛаТ, АСаТ, ЖБ – ны көбеюін тоқтатты. Зерттеу тобында салыстырмалы топқа қарағанда ДК- ті - 2,7 есе тоқтатты және бақылау тобына қарағанда -3,8 есе, ШО-ның жиналуын шектеді.

Түйінді сөздер: Цитизин амидофосфанаты, «Эссенциале» гепатопротекторы, липидтердің асқын тотығуы, төртхлорлы көміртек, гепатопатия.

Introduction. According to WHO data acute exogenous poisonings composes more than a third “the ecological diseases” [1-3]. About 100 thousand of different substances are constantly in the process in production and in everyday life, that are used as medicines. It inevitably leads to growth of number of acute poisonings around the world. One of frequent complications of acute exogenous poisonings is toxic hepatopathy. There are 3 main mechanisms of hepatopathy development: decrease of specific function of hepatocytes, disturbance of regional microcirculation and bile secretion [4,5]. The great significance has been given to the hyperoxide oxidation of lipids (HOL) when acute intoxications and the role of its disturbances in the genesis of complications in recent years, including toxic hepatitis. Entering of toxic substances into organism and their subsequent metabolization leads to increasing formation of free radicals.

So, in the process of biotransformation of xenobiotics while the action of microsomal monooxigenases high-toxic superoxidic anions due to decomposition of oxygenated ferrocomplexa citochrome P-450 [1] are formed. Formation of the active forms of oxygen (AFO) in a liver during the work of cytochrome of P-450-dependent system of microoxygenases of microsomes - continuous physiological process [2,3], however in conditions of affect of toxic agents AFO production sharply increases in conditions, and there is a threat of desease of the whole organism. The identification of HOL role in pathogenesis of toxic hepatitis at acute exogenous poisoning led to search of new effective medicine with antioxidatic properties. As hepatoprotecting means when experimental toxic hepatitis of different types vitamin E and Selen containing preparations, ubiquinone-9, Silibininum, sodium selenit and polyphenolums, Eplir, Essentiale, Maksar, Legalonum and a number of other substances were studied. Inhibiting free radical oxidation, antioxidants protect cellular membranes from damage and prevent death of hepatocytes [4,5].

In this regard the purpose of our researches is comparative studying of the anitioxidant properties of “Essentiale” and amidophosphonata citizina of classical model of acute toxic hepatitis, caused by four-chloride Carbonium.

Materials and Methods. All experimental procedures were carried out according to the requirements of GLP [6]. As it is known that disintegration of tetrachlormethane (CCL_4) leads to formation of the free radicals initiating reactions HOL. The poison with obvious pro-oxidatic action and hepatotoxic effect is the most suitable model for check of various medicines for antioxidatic and cytoprotective activity.

Acute toxic hepatitis was caused in 24 rats by means of intraperitoneal introduction of 40% oil solution CCL_4, in the dose of 0,2 ml / 100,0 once. An hour before CCL_4 injection the animals of tested group received amidophosphanate citizina in the dose 100 ml/kg day, while the animals of comparison group - “Essentiale” 50 ml/kg/day, the control ones - equal quantity of normal saline solution.

For biochemical researches and researches of the system HOL - AOP the blood from the central vein in the course of decapitation was tested. In blood serum the level of the general bilirubin (TB), protein (TP), aminotransferases (AlAT, AsAT), the alkaline phosphatase (AP) was determined by the standard technique [1,5,7]. The condition of the system HOL-AOP was estimated by the contents of products of lipohyperoxidation in the erythrocytes by the method of VN Ushkalova and ND Kadochnikova [8]. For integrated determination of the level of total primary (TPP) and secondary (TSP) products of HOL, as well as the Schiff Bases (SB)

methodical approach of EI Lvovskaya was used [9]. The activity of catalase (CT) in plasma was estimated according to the speed of destruction of hyperoxide of Hydrogen and expressed in seconds, in the erythrocytes - by method of MA Korolyuk and coworkers [10]. In the erythrocytes the level of the average molecules (AM) was determined by the method of AI Kovalevskaya and OE Nifantyev [11].

Statistical processing of the obtained data is carried out on the personal computer by the method of variation statistics with use of the package of application programs (SAS version 9.0 and JMP version 5.0 (SAS Institute Inc., Gary, NC)). The differences of the compared values were considered to be reliable at the value of reliability criterion (t) of Fischer – Student, equal to > 2.

Results. Use of amidophosphanat citizina 1 h before poisoning with tetrachlormethane led to even more positive shifts in biochemical indicators, characterizing liver function. In the tested group unlike the group of the animals taking “Essentiale” there was a distinct reduction of activity growth of AlaT, AsaT, amount of TB in the blood. However the level of activity APh was slightly higher, than in the group of comparison (table 1).

Table 1 - Biochemical indicators of blood when experimental toxic hepatopathy of rats and its correction by hepatoprotectors (Хұm)

Indicators	Intact group	Thecontrol group	Experiment-ed group	Compari-son group
AlaT, mmol/l	306±4,13	1081±239	745±65,4	899±76,7
AsaT, mmol/l	263±20	406,5±41,5	293,2±28,7	320±31,1
Total Protein, g	71,6±3,0	66,3±4,4	70,7±0,96	66,3±1,3
APh, cond.units.	87,0±2,3	150,4±3,0	132,2±4,63**/***	110,2±2,6
TB, mkmol/l	13,4±2,3	27,2±3,0	16,74±2,66	19,5±0,85
Bayesia	Bayesia ltd	Yes	Free	Chain diagrams
Hugin Expert	Hugin	Yes	Commercial	Chain diagrams
Netica	Norsys[9]	Yes	Commercial	Oriented
BNet	Murphy (U.C.Berke-ley)[10]	Yes	Free	Oriented

* P<0,05 between I and II; ** P<0,05 between II and III; *** P<0,05 between III and IV

It is known that the effect of chlorinated hydrocarbons causes sharp induction of HOL and disbalance of system of antioxidatic protection (AOP). Poisoning with four-chloride Carbonium led to growth of the diene conjugates (DC), cetodienes (CD), the total primary products (TPP), the total secondary products (TSP) and final products (FP) of lipohyperoxidation, and indicators HOL exceeded the control several times (table 2). So, the increase of contents of DC 2,3 times, CD 2,6 times, SB 2 times that testifies about deviations of oxidizing metabolism and practically out of control chain reaction of lipohyperoxidation.

Table 2 - Indicators of HOL-AOP system in the blood of rats while experimental hepatopathy and correction by hepatoprotectors (X±m)

Indicators	Intact group	Control group	Compari-son group	Experimen-tal group
DC.cond.units/ml	20,1±4,8*	78,6±9,4	55,04±6,0**	20,6±1,5***
CD.cond.units/ml	7,2±0,21 *	13,8±2,57	11,5±3,4	8,55±2,4***
TPP. cond.units/ml	0,39±0,03*	0,48±0,024	0,11±0,035**	0,12±0,04***
TSP. cond.units/ml	0,124±0,04*	0,82±0,13	0,39±0,18**	0,36±0,072***
SB.	0,066±0,012*	0,137±0,025	0,155±0,026	0,11±0,019
CTp. sec.-1	0,037±0,003	0,039±0,001	0,038±0,06	0,04±0,003
CT e.molH20. ml er/min.	0,079±0,008*	0,029±0,0025	0,033±0,003	0,027±0,005
CM. cond.units/ml	0,06±0,003*	0,079±0,003	0,059±0,001**	0,05±0,001***

* P<0,05 between 1and II; ** P<0,05 between II and III; *** P<0,05 between II and IV

Preventive introduction of amidophosphanat citizina also influenced HOL system - AOP is positively that was comparable to antioxidate action of “Essentiale”. Amidophosphanat citizina kept the growth of DC in more active and distinct way (level the diene conjugates in experienced group 2,7 times lower, than in the group of comparison, and 3,8 times than in the control group) and limited accumulation of SB. On other positions essential differences are not noted, however amidophosphanat citizina reduced the activity of HOL much more significantly.

Discussion. Acute poisoning with four-chloride Carbonium leads to development of toxic hepatitis [4]. It is proved by sharp increase of contents of transaminases, in particular ALT that is rather characteristic of toxic contamination of liver, and also rising of level of the total bilirubin

and activity of alkaline phosphatase. Intensity evidence of 2 leading syndromes: cytolytic and cholestatic that mean serious damage of liver that then was confirmed morphologically.

It is necessary to assume further natural development of the syndrome of hepatocellular failure [5]. Preliminary introduction "Essentiale" into the tested animals in general gave positive result though in the group of comparison indicators of aminotransferases, TB and APh were significantly lower than in the control group. This preparation due to the direct hepatoprotectingaction (essential phospholipids) weakens the development of cytolytic and cholestatic syndromes.

Introduction of "Essentiale" 1 h before poisoning positively affected the general picture of the complex multiphase HOL-AOP system. Use of this preparation caused less growth of toxiferous metabolites of lipohyperoxidation at all levels: DC, CD, TPP, TSP, TB (table 2). The activity of CT in erythrocytes increased slightly and the AM level, the indicators of intoxication and the hyperoxide-induced proteolysis decreased. In the whole "Essentiale" stops HOL, stabilizes membranes and can be considered as antioxidant.

The expected growth of the activity of one of the key AOP CTkey enzymes didn't happen, and in erythrocytes this indicator even decreased a little. Evidently, this enzyme of the antihyperoxide, second line of antioxidatic protection is not effective in the conditions of excess of hyperoxide of Hydrogenium.

Amidophosphanat citizina doesn't refer to any known classification of antioxidants, composing 7 groups of the preparations inhibiting free radical oxidation (FRO).

Though 2 basic positions about the mechanism of action of antioxidants are recognized, FRO inhibitors compose not only the true antioxidants interacting with lipide radicals but also many other compounds braking HOL by influence on one of its links. This effect can be also implemented through influence on various processes closely connected with lipohyperoxidation: the hydrolysis of lipids, synthesis of Prostaglandinums and leukotrienes, enzyme systems producing AFO can be the result if not direct, then indirect action [1,5].

Conclusions. New domestic hepatoprotector amidophosphanate has antioxidatic properties that is confirmed by expressed HOL inhibition, initiated by four-chloride Carbonium. It is possible that the mechanism of hepatoprotecting action of amidophosphanata citizina is bound with antioxidatic and membrane stabilizing effect. The mechanism of preparation

effect is not found out completely that assumes further studying of this perspective hepatoprotector including clinical conditions.

References

1. *Nema A.K., Agarwa A., Kashaw V.* Hepatoprotective activity of Leptadeniareticulata stems against carbon tetrachloride-induced hepatotoxicity in rats //Indian. J Pharmacol. - 2011. - №43(3). –P. 254-257.

2. *Omar T.Y.* Protective efficacy of Glycyrrhizaglabra on CCI4-induced liver injury in rabbits // World. J Pharmtyi Res. - 2014.- № 3(3).- P.3627-3638.

3. *Rahmat A.A., Dar F.A., Choudhary I.M.* Protection of CCI4-Induced Liver and Kidney Damage by Phenolic. Compounds in Leaf Extracts of Cnestisferruginea (de Candolle) // Pharmacognosy Res. - 2014.- №6(I).- P.19-28.

4. *Al-Duais A.M., Al-Awthan Y.S., Mukhtar A.A., Shamsan A.A.* Prevention of Carbon Tetrachloride (CCI4)- Induced Liver Damage in Guinea Pigs by Cyphostemma Digitatum. // Life Scis. - 2012. -№6.- P.137-143.

5. *Duanbekova G.B., Issabayev A.S., Karynbayeva M. Zh. et al.* Delection of side effects of pharmacological compounds 0.0 - dimethyl of cytidine diphosphate.- Messenger KRSU. – Bishkek, 2017. - №3 (7). - P.172-174.

6. Standard GLP - ГОСТР-53434-2009: «Principlesof Laboratory Practice».

7. *Duanbekova G.B., Baikenova G.G., Issabayeva G.M. et al.* Toxicity studies with long-term administration of pharmacological compounds amidophosphate cytosine – Messenger KRSU. - Bishkek, 2017. - №3 (17) - P.161-164.

8. *Ushkalova V.N., Kadochnikova G.D., Vladimirov V.A., Archakov A.I.* The way of definition of products of hyperoxide oxidation of lipids in blood //N: Nauka,1972. - 237 p.

9. *Korolyuk M.A., Ivanova L.I., Mayorova I.G., et al.:* Method of definition of activity of catalase //Lab Work, 1988. - №10.- P.15-17.

10. *Kovalevskiy A.N., Nifantyev O.E.* Remarks by screening method of definition molecules of average weight // Lab Work, 1989. - №10. – P.35-37.

11. *Kamyshnikov V.S.* Methods of clinical laboratory researches.- 4th ed. –M.:MEDpress-Inform, 2011. – 752 p.

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