HEPATOPROTECTION AGAINST CHEMICAL INFLUENCE: COMPARATIVE EFFECTS 5-HYDROXY-6-METHYLURACIL (OXYMETHYLURACIL), COMPLEX COMPOUND «OXYMETHYLURACIL + SODIUM SUCZINATE» AND SILIMARINE

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ABSTRACT
Experimental data on comparative hepatoprotective activity of 5-hydroxy-6-methyluracil (oxymethyluracil), complex compound «oxymethyluracil + sodium suczinate» and referent hepatoprotector silimarine have been analyzed and systematized. On the models of liver affection by industrial toxicants – tetrachlormethane, dichlormethane, PCB-containing drug "sovtol-1", 2,4-dichlorphenol, trichlormetafosis and ethanol it was established that oxymethyluracil has a pronounced hepatoprotective influence comparable in its effectiveness with that of silimarine and excels it on the models of liver affection by dichlormethane, trichlormetafosis and ethanol. On the model of liver affection by combination of sovtol and ethanol complex compound «oxymethyluracil + sodium suczinate» proved effective. The data obtained allows to make the conclusion that oxymethyluracil is the drug for which hepatoprotective activity may have an independent clinical significance.

KEYWORDS — hepatotoxicants, hepatoprotectors, toxic hepatopathy, toxic hepatitis, cirrhosis of liver, effectiveness index, oxymethyluracil, silimarine, fermentative markers, functional markers.

1. OXYMETHYLURACIL: ACTIVE MECHANISM AND PHARMACOLOGICAL PROPERTIES

Oxymethyluracil 5-hydroxy-6-methyluracil stimulates the immunity, regenerative processes, has anabolic and anticatabolic effect, activates bioenergetical processes, some ferments of antioxidant protection, suppresses alteration and exudation, regulates the processes of lipids peroxide oxidation, stabilizes cell and organelle membranes, intensifies ATPase activity, performs the function of «radicals trap», protects the biostructures against active forms of oxygen and toxic peroxide compounds. The drug has antioxidant activity, stimulates nonspecific resistance of organism, exerts nootropic, cardio-protective, stress-protective and demethemoglobinising influence (table.1). Previously oxymethyluracil proved to have therapy and preventive effect after bad acute poisoning by chemical substances having neuro-toxic, hemo-toxic and hepato-toxic effect. The drug revealed positive effect being applied as a corrective remedy against side effects of cholinolitic drugs, cholinesterase reactivators, strofantine, digoxine, corasole, etc [4,10].

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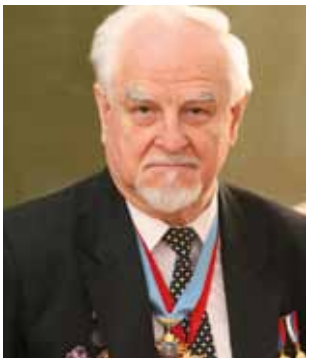

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OXYMETHYLURACIL HAS IMPORTANT PHARMACOLOGY PROPERTIES [4], OXYMETHYLURACIL:

- low-toxic drug without allergic, mutagenic and carcinogenic effect;
- no arrhytmogenic or negative effect at the heart conductive system,
- no general toxic effect;
- when injected in enteral or parenteral way it produces cardiotonic effect, does
- not change the heart rate;
- eliminates depression of heart rate function on the models of experimental myocardial infarction caused by left coronal artery blocking and theophylline-adrenaline myocarditis;
- in therapeutic doses does not change the left ventricle rate phase structure, in two or three times higher doses prolong expulsion phase, shorten

Table 1. The Action Mechanism and Pharmacology Characteristics of Oxymethyluracil [2–11]

1. Possible primary action mechanism: Free-radical oxidation inhibition RNA-polymerase activation Right protective influence on membrane Uridinephosphatase blockade **2. Protective and recovery mechanism:** Protection against active forms of oxygen and peroxide compounds Activation of antioxidant ferments (catalyze, SOD) activity Stabilization of cell, subcell membranes Rise of ATPase activity Rise of SDG , NADN-Dg activity Activation of adaptive synthesis of PNA and proteins Alteration and exudation suppression Reparation processes stimulation Demethaemoglobinising effect Rise of cAMPH	**3. Pharmacology effects spectrum:** Antioxidant effect Immunity stimulating effect Antitoxic effect Antiseptic effect Anabolic effect Anti-catabolic effect Rise of the organism non-specific resistance Hepato- and pancreas-protective effect Stress-protective effect Radio-protective effect Membrane-protective effect antispasmodic activity cardioactive effect nootropic activity actoprotective effect

isometric contraction phase with dogs;
- does not change arterial or perfusion pressure under intravenous injection with cats;
- does not influence the bronchus tone or bronchus spasm caused by carbacholine and proserine;
- has some antispasmodic effect on smooth musculature of intestine;
- shortens the latent period of motor-defense conditioned reflex with rats and inhibits its extinction;
- rises motor activity of rats and mice, prolongs the duration of rats' stereotype behavior caused by injection of phenamine;
- rises actoprotective activity of ethimizol, inhibits the extinction of chronic fatigue;
- does not change the coordination of movements;
- does not cause pronounced biochemical changes or changes in blood cell structure;
- compatible with a lot of drugs, used in complex therapy of intoxication (including cholinolitics, cholinesterase reactivators, methylene blue, cystamin, etc.)

Oxymethyluracil and oxymethyluracil sodium suczinate were synthesized by Ch.D. Krivonogov V.P. in Organic Chemistry Institute of Ufa Science Center of Russia Academy of Science.

According to the Order N 302 from July 29, 1996 of Minister of Health and Medical Industry of the Russian Federation the oxymethyluracil drug is allowed for medical practice and industrial output [13].

2. MODELS AND METHODS OF INVESTIGATION

Damage of liver was modeled by injections of hepatotoxicants to white he-rats in the following doses:

tetrachloromethane: 2 ml per 1kg of the body weight every other day within 30 days period;

dichloroethane: 0.01 DL/50 during 3 weeks in 10% olive oil solution;

sovtol-1: 0.25 ml per 100g of the body weight twice a week within 28 days + 10% ethanol solution for drinking (Patent of the Russian Federation № 2197018 from 16.02.2000);

2,4-dichlorphenole: 400 ml per 1kg of the body weight (0.8 DL/50);

ethanol: 7 ml per 1kg of the body weight daily within 30 days period;

trichlormethafos: 47 mg per 1kg (0.2 DL/50) daily within 28 days period.

Complex of biochemical investigation included total protein determination (g/l), cholesterol (in mmole/l), triglyceride (in mmole/l), bilirubin (in mcmole/l), ferments activity: urokaninase (UrN) (in nmole/t.s.), alaninaminotranspherase (AlAt in mmole/t.s.), aminotranspherase aspartat (AsAt in mmole/t.s.) and alkaline phosphatase activity (AP in mmole/t.s.).

Biochemical investigation of blood serum was carried out with biochemical analysator «Encore» (Austria). The estimation of oxymethyluracil and comparative drug (cilimarine) hepatoprotective effect was carried out by defining the index of effectiveness of hepatoprotective effect of investigated drugs – EI (in %) – difference in shares between index of liver damage level in the control group and in the group of animals, which took the drugs under investigation. EI of hepatoprotective effect was determined according to the following formula:

$$EI = (Ic - Ie)/Ic \cdot 100$$

where Ic and Ie — mean values of indices in control and experimental groups correspondingly.

EI was calculated separately according to functional indices data (general bilirubin, total protein, cholesterol and triglycerides) and to liver damage fermentative markers indices (UrN, AlAt, AsAt, AP).

EI positive meaning (plus-effect) points to damage index decrease.

EI negative meaning (minus-effect) points to damage index increase.

The estimation of results was made with the help of Student-Fisher parametrical test.

Oxymethyluracil and cilimarine were injected in equal doses of 50 mg/kg intra-gastric1,5 hours before toxicant.

3. HEPATOPROTECTION WITH THE USE OF OXYMETHYLURACIL, COMPLEX COMPOUND «OXYMETHYLURACIL + SODIUM SUCZINATE» AND SILIMARINE

Since the time of the first pyrimidines introduction into the practical medicine there were attempts to use them as hepatoprotectors. New pyrimidine derivative – oxymethyluracil attacted our attention due to its distinct antioxidant properties, which were revealed in V.A.Myshkin's special investigations [2,7].

It is stated that oxymethyluracil does reveal itself as hepatoprotector on the models of liver affection by industrial toxicants [models 1, 2, 3, 4, 6], as well as ethanol [model 5], (table 2). It reveals itself in real decrease of marked ferments of citolyse and cholestasis level, and also in normalization of bilirubine, cholesterol and triglycerides level. Rise of total protein level in the blood serum indicates the preservation of protein synthesizing liver function under oxymethyluracil. Silimarine proved less effective in this connection on the models of liver affection by tetrachloromethane, sovtol-1 and trichlormethafos, that is confirmed by corresponding EI indices EI (Table 2).

The comparison of oxymethyluracil and silimarine hepatoprotective effect EI drives us to the conclusion that oxymethyluracil does not yield to silimarine in its ability to normalize biochemical indices in rats' blood serum, and consequently the functional and metabolic state of liver when it is damaged by the investigated hepatotoxicants.

The results of previously carried out morphometrical and histachemical investigation of rats' liver correspond to biochemical data and indicate of much less degree of necrotic changes, lipidosis and granular dystrophy with rats which was given oxymethyluracil along with intoxication by 2-dichloroethane, tetrachloromethane sovtol-1, 2,4-dichlorphenole, orthochlorphenole and ethanol [2, 5, 12], beam-like structure of the organ is better preserved and activation of liver regenerative processes is more pronounced.

Specialists in the sphere of toxicology and ecopathology may take interest in the data on oxymethyluracil hepatoprotective effect (OMU) acquired during the experiments on the models of liver affection with PCB-containing drug "sovtol-1" and «sovtol-1 + ethanol» composition (Table 2, 3). Nowadays over 200 000 transformers and condensers containing about 18 000 tons PCB oils [15] are known to be in exploitation and in reserve.

Table 2. *Indices of effectiveness (EI) of oxymethyluracil (I) and silimarine (II) hepatoprotective effect calculated according to fermentative and functional markers of liver damage caused by hepatotoxic substances**

N	Hepatotoxicants	EI (%)			
		Fermentative markers		Functional markers	
		I	II	I	II
1	Tetrachloromethane	+29.9	+36.2	+33.2	+27.6
2	2- dichloroethane	+38.9	-	+41.0	-
3	Sovtol-1	+15.47	+11.7	+24.4	+5.8
4	2,4-dichlorphenole	+16.02	-	+17.8	-
5	Ethanol	+28.5	+15.5	+14.4	+15.7
6	Trichlormethafos	+33.3	+26.2	+35.9	+9.8

Note: ** — Calculated according to data [2, 3, 4, 5, 6, 7, 8, 9, 11]*

Table 3. *Hepatoprotective effectiveness indices (in%) of complex: «oxymethyluracil + sodium suczinate» (I), oxymethyluracil (II) and sodium suczinate (III), calculated according to functional markers of liver affection by PCB-containing drugs «sovtol-1» and «sovtol-1 + ethanol» [8, 14].*

Hepatotoxicants	EI (%)		
	I	II	III
Sovtol-1	+ 33.13	+ 19.9	+ 5.7
Sovtol-1+ethanol	+ 29.3	+ 12.6	+ 3.9

On the models of liver affection with PCB-containing drug "sovtol-1" and «sovtol-1 + ethanol» composition there was investigated the effect of complex drug: «oxymethyluracil + sodium suczinate» which was synthesized in OCI of RAS Ufa Scientific Centre.

Sovtol poisoning of animals causes acute affection of liver, its main symptoms are cytolysis, fibrosis and cirrhosis. In histologic liver drugs the fibrosis fields occupies 3,1% of environment (against 0,5% with healthy rats). Hepatocytes are located mainly in periportal zone, which is in the state of protein and lipid dystrophy. Decomplectation of liver beams and proliferation of connective tissue in portal tract with fibrous bands deep into lobes can be determined. In the field of vi-

sion there are a lot of necrotized hepatocytes, oedema and stroma loosening; in cellular infiltrate there are lymphocyte clusters.

Morphometry of liver drugs revealed lowering of nuclear-cytoplasmic ratio of stroma to parenchyma.

There were revealed pronounced metabolic and functional problems: the rise of concentration of POL-level products of isolated double links, diene conjugates, triene conjugates, and also glycosaminoglycan in liver tissue. Besides the concentration of oxyprolin in acid soluble and acid fast collagen fractions increases. In blood serum there is revealed the rise of activity of marked urokaninase ferments, AlAt, AcAt, acid phosphatase, alkaline phosphatase and lactic dehydrogenase [6, 9].

Under the influence of oxymethyluracil and especially «oxymethyluracil + sodium suczinate» complex the liver architectionics was obviously improved: the lobes beam-like structure and their right radial direction was partially restored. The level of dystrophy became less distinct: clusters of glycogen containing lobes were preserved, lipid dystrophy was less pronounced, though the signs of hepatocytes protein dystrophy remained, they were not so distinct as in control.

Judging to the effect on the value of indices which reflect the functional state of liver (general bilirubine, total protein, cholesterol, triglycerides) the maximum effect was achieved at using «oxymethyluracil + sodium suczinate» complex, when effectiveness index was + 33.13%, less pronounced results were achieved at using oxymethyluracil (+ 19.9%) and especially sodium suczinate (+ 5.7%), On the model of liver affection by «sovtol + ethanol» the maximum hepatoprotective effect was achieved at using the oral «oxymethyluracil + sodium suczinate» complex and was only 29.3%. The effect of oxymethyluracil and suczinate was still lower and made 12,6% and 3,9% accordingly [table 3].

We believe that using of oxymethyluracil together with the known hepatoprotectives aimed at increasing their effectiveness is not deeply investigated but rather perspective as OMU rises the effectiveness of many famous drugs and at the same time lessens their toxic effect, undesirable consequences and rises tolerance [4,10]. In conclusion we present Table 4 in which we tried to systematize our aspect of this problem.

Table 4. *Empirical expediency of oxymethyluracil and hepatoprotectives combination in case of liver toxic affect*

Hepatoprotectives	Oxymethyluracil
Drug of animal origin (hepatosan)	–
Artichoke leaves extract	±
Tykveol	±
Liv 52	±
Essenciale phosphor lipids	+
Lipoic acid	+
Ursofalk	+
Hepa-maerz	+
Lactulose	+
Heptral	+
Metadoxile	±
Hepabens	–

Note: *+ combination is possible*
± expediency of combination is doubtful
– combination is not expedient

CONCLUSIONS

1. Oxymethyluracil shows a pronounced hepatoprotective effect on the models of liver affection with tetrachloromethane, dichlorethane, ethanol, trichlormethafos.
2. Hepatoprotective effect of oxymethyluracil on the models of liver affection with tetrachloromethane, dichlorethane, ethanol, trichlormethafos and also PCB -containing drug «sovtol-1» is revealed in prevention of hyperfermentation, normalization of bilirubin, cholesterol, total protein and triglyceride level.
3. Oxymethyluracil is not inferior to standard hepatoprotective silimarine in its pronounced hepatoprotective effect on the models of liver affection with tetrachloromethane sovtol-1, ethanol and trichlormethafos.
4. Complex compound: «oxymethyluracil + sodium suczinate» has more pronounced hepatoprotective effect than oxymethyluracil and sodium suczinate on the models of liver affection with PCB -containing drug sovtol-1 and its combined affection with «sovtol-1 + ethanol».

PRACTICAL RECOMMENDATIONS

1. The using of oxymethyluracil as hepatoprotective is approved in the cases of subacute intoxications with tetrachloromethane, 2-dichlorethane, sovtol, ethanol, trichlormethafos, and also in the cases of acute intoxication with 2,4-dichlorphenole.
2. Investigations of hepatoprotective effect of oxymethyluracil combinations with suczinate-containing drugs as well as with drugs having presumably detoxical effect like hepa-maerz, glutargine, ademethionine, metadoxile, etc. can be considered reasonable.

3. Investigations on synthesis of new oral and parentheral complex compounds of oxymethyluracil and suczinate can be considered perspective.

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