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Cytisin Amidophosphate – The New Cytoprotector

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ABSTRACT

This article describes the results of a clinical study of O, O dimethyl-N-cytinizylphosphate in healthy volunteers and 142 patients with the verified diagnosis of acute toxic hepatitis. The tolerability was good. The efficiency of the drug is considered to be very high, normalization of the state took less time in comparison with standard treatment; a significant improvement was achieved immediately after the first injection.

Key words: *Cytoprotector, Acute toxic hepatitis*

Acute toxic hepatitis is a quite common pathology of the liver, frequently caused by drugs or alcohol[5,6)]. Acute toxic hepatitis is the second most common cause of liver morbidity in Kazakhstan, viral hepatitis is the most common (371 patients in Kazakhstan during 2010)[10)]. Acute alcohol-related hepatitis is a severe liver disease with a high death rate (mortality can reach 50%)[9)].

The problem of effective and rational treatment of non viral hepatitis is still unresolved[3)]. The majority of clinical studies in evidence-based medicine do not lead to effective methods of therapy. The exception is treatment using corticosteroids, but this method is not sufficiently effective (mortality in severe forms remains about 30% during the next 2 months)[14,17)]. Despite the lack of uniqueness in the evaluation of clinical effect, the testing of the effects of antioxidants and other hepatoprotectors on acute toxic hepatitis continues, in the hope of finding the optimal variant of the therapy[1,12,15)].

However, there is still a hope that using drugs will lead to the acceleration of the regeneration of cells (hepatocytes in particular) or reduction of the damage from hepatotropic poisons[2,7,8)].

During past several years screening of the bioactivity of a list of semi-synthetic derivatives of alkaloid cytosine has been performed in our laboratories. The result was the synthesis of O,O dimethyl-N-cytinizylphosphate (cytisin amidophosphate), which is a phosphor-derivative of alkaloid cytosine[16)].

The main therapeutic properties (antioxidant, membrane stabilizing, cholagogic) were identified during the preclinical studies. Cytoprotective properties were noted in the following clinical situations: a) acute toxic hepatitis caused by ethyl alcohol, acetaminophen, tetrachlorethylene, acrylic alcohol, tetracycline; b) chronic hepatitis caused by barbiturates and tetrachlorethylene; c) jaundice. It was also proved that the substance accelerates the regeneration of liver tissue after 2/3 resection. The level of preventive and curative effects of the substance is higher than that of comparator drugs (Essentiale, Silibinin). Increasing cholinesterase in the blood (if the drug is taken for more than 2 weeks), raising blood pressure and accelerating cardiac rhythm (if taking large doses at one time) are the only toxic effects of the substance.

Currently, this pharmacological compound is allowed in Kazakhstan for clinical trials under the name of Cytaphat ("Цитафат").

MATERIALS AND METHODS

The object of research - O,O dimethyl-N-cytinizylphosphate under the name of Cytaphat (the chemical formula is described in Fig. 1).

The synthesis was carried out by a group of researchers at the Science and Research Institute of Organic Synthesis and Coal Chemistry under the guidance of A.M. Gazaliev.

142 patients (age: 16 - 56) with the verified (by history, objective status, ultrasound and biochemical tests) diagnosis of acute toxic hepatitis (poisoning with ethyl alcohol – 49, alcohol surrogates - 89, paracetamol - 3, reserpine - 1), who underwent treatment in the toxicological department were included in the clinical research. The patients were randomly divided into 3 groups: 1) 34 patients were taking Essentiale (200 ml of 5% glucose + 10 ml of Essentiale) intravenously once per day for 3 days; 2) 94 patients were taking Cytaphat (10 mg of the drug per 1 kg of weight +

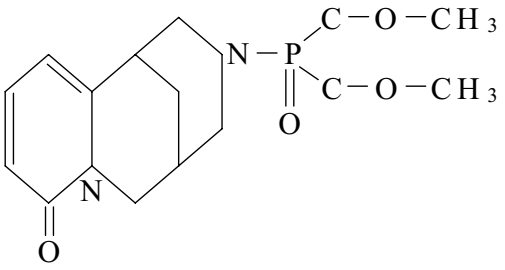

Fig. 1. The structural formula of O,O dimethyl-N-cytinizylphosphate

200 ml of 5% glucose) intravenously once per day for 3 days; 3) 14 patients were taking a placebo (10 mg of placebo + 200 ml of 5% glucose) intravenously once per day for 3 days. In order to simplify the analysis, they used benchmarks of the activity of ALT (alanine transaminase), AST (aspartate transaminase) and total bilirubin in the blood of 47 (22 males and 25 females) students of the medical university. The result was considered as normal.

The method of clinical trials was double-blind. The activity of markers of cytolysis was determined by the level of ALT, AST and total bilirubin on a semi-automatic biochemical analyzer[11].

Parameters describing the system of lipoperoxidation and antioxidant system in the blood (plasma, red blood cells) were the following: 1) primary products – conjugated dienes, ketodienes, summary primary products (SPP); 2) secondary products – malondialdehyde, summary secondary products (SSP); 3) final products – Schiff base. The state of the antioxidant system was evaluated by catalase and glutathione peroxidase[4].

The results were processed by the method of variational statistics[13]. Two sample T-tests were applied to assess the reliability of the results.

The study was discussed by the Center for Life Sciences Ethics Committee on September 8, 2011 (protocol #2). After careful consideration, the Center for Life Sciences Ethics Committee approved the study.

RESULTS AND DISCUSSION

Preliminary research on healthy volunteers revealed that 200 mg of the drug/kg is the maximum tolerable intake. Side effects when taking high doses (100-200 mg/kg) are tachycardia and hypertension.

At a dose of 100 mg/kg, 3 of 27 volunteers noted a high heart rate which disappeared in 15-20 min, and one person had a burning sensation in the vein. Only one probationer experienced hypertension: before drug administration - 115/70 mmHg (average of three measurements within 30 min), 15 min after the injection - 135/90 mmHg, 30 min - 130/75 mmHg, 60 min - 120/70 mmHg, day after the injection - 125/80 mmHg, 10 days - 115/75 mmHg (average of three measurements). The respiration rate did not change. There were no changes on the ECG. All laboratory tests were within normal limits. At a dose of 200 mg/kg all 3 volunteers felt cardiopalmus. The objective data is shown in Table 1.

Initially, in all cases, cytolytic syndrome was confirmed by biochemical tests of AST and ALT in the presence of moderate cholestatic syndrome (maximum bilirubin - 59.1 mmol/liter). Ultrasound examination in all cases revealed diffuse changes of the liver tissue, in some cases they were accompanied by the phenomena of hepatomegaly and reactive pancreatitis.

3 days after the implementation of Essentiale or Cytaphat all patients reported a significant (subjective opinion) recovery: reduction of the discomfort, epigastric pain and pain in the right upper quadrant; normalizing of the appetite; and feeling active and vigorous.

Patients who received a placebo, subjectively felt slightly better, but a positive trend was almost absent. 8 of 14 patients showed no positive changes during the first 3 days.

The dynamics of indicators characterizing the level of cytolysis are presented in the Tables, separately for each group.

Dynamics of the basic indicators with the comparator drug are shown in Table 2.

Two patients were dropped out of this group. The first patient had a strong urge to vomit and he had repeatedly vomited after the first injection of Essentiale. The patient's state normalized after the drug's removal. A technical error occurred in the second case. As a result, Essentiale was infused only once, whereas 3 infusions were required according to the research protocol. This result was not taken into consideration.

Table 3 shows changes of the biochemical

Table 1. Pulse rate and blood pressure after administration of Cytaphat at a dose of 200 mg/kg.

Probationers	Pulse rate: initial, 15, 30, 60 min after the injection	Blood pressure: initial, 15, 30, 60 min after the injection
1	58 – 96 – 64 – 56	130/75 – 140/90 – 140/90 – 130/70
2	68 – 112 – 90 – 62	110/70 – 115/70 – 130/85 – 115/70
3	72 – 90 – 86 – 66	115/70 – 140/75 – 120/70 – 120/70

indicators with the Cytaphat injection.

Three patients withdrew from the research. The day after the first injection of Cytaphat two patients' blood showed an increase of ALT (1168 nmol/(sl)), though both felt better in general. Later the cytolytic syndrome has subsided (decrease of ALT) and the patients were discharged in a satisfactory condition.

One subject (female, 58 years old, rheumatic fever in history, without the formation of a cardiac anomaly) left because tachycardia (120 bpm) evolved after the second infusion of the examined drug. The injection of the drug was discontinued. This led to normalization of the patient's state in 30 min. 6 days after admission, she was discharged in a satisfactory condition without any negative consequences of this episode.

Table 4 shows changes in the biochemical indicators among the subjects who were injected with a placebo.

It has been shown that Cytaphat has a positive clinical effect on all types of toxic hepatitis. This is different from the effect of the placebo and better than the effect of the comparator drug.

A strong decline in cytolytic syndrome (indicators: ALT, AST, alkaline phosphatase, thymol), reduction of the cholestatic syndrome (decrease of the bilirubin) and indicators of the system of

Table 2. Patients' biochemical indicators with comparator drug (Essentiale) (average ± standard deviation of the average).

Groups	Biochemical indicators of cytolysis		
	ALT nmol/(sl)	AST nmol/(sl)	Bilirubin mcmol/liter
Desirable	28 – 125	28 – 189	8.5 – 20.5
Before treatment (n=34)	542.2 ± 38.2	298.2 ± 36.1	54.3 ± 6.1
After treatment (n=32)	232.2 ± 20.8	154.7 ± 19.6	18.6 ± 2.4
p (between commencement and completion of the treatment)	< 0.05	< 0.05	< 0.05

Table 3. Patients' biochemical indicators with Cytaphat (average ± standard deviation of the average).

Groups	Biochemical indicators of cytolysis		
	ALT nmol/(sl)	AST nmol/(sl)	Bilirubin mcmol/liter
Desirable	28 – 125	28 – 189	8.5 – 20.5
Before treatment (n=94)	458.6 ± 27.4	347.5 ± 40.2	38.8 ± 2.1
After treatment (n=91)	248.9 ± 35.8	109.1 ± 8.7	19.8 ± 1.7
p (between commencement and completion of the treatment)	< 0.05	< 0.05	< 0.05

Table 4. Patients' biochemical indicators with placebo (average ± standard deviation of the average).

Groups	Biochemical indicators of cytolysis		
	ALT nmol/(sl)	AST nmol/(sl)	Bilirubin mcmol/liter
Desirable	28 – 125	28 – 189	8.5 – 20.5
Before treatment (n=14)	418.5 ± 30.7	322.6 ± 18.4	40.1 ± 6.2
After treatment (n=14)	354.7 ± 25.0	297.1 ± 33.6	34.5 ± 3.7
p (between commencement and completion of the treatment)	≤ 0.05	> 0.05	> 0.05

lipoperoxidation were registered in the 24 hr period after the injection. The next day ultrasound showed positive changes in all cases (reduction of diffuse changes and effects of reactive pancreatitis), and hepatomegaly disappeared.

The differences in activity levels of indicators of cytolysis can be seen in Table 5.

As can be seen from the data, the effect of Essentiale on biochemical parameters (AST, ALT and bilirubin) was different from the effect of the placebo when given to patients with acute toxic hepatitis. Cytaphat also had a positive effect distinguishable from that of the placebo.

Statistically significant differences in the dynamics of the indices of cytolysis between the new pharmacological compound, Cytaphat, and a standard hepatoprotector, Essentiale, among patients with acute toxic hepatopathy were not found.

The concentration of primary (conjugated dienes, ketodienes, SPP), secondary (malondialdehyde, SSP) and final (Schiff base) products of the system of lipoperoxidation in the blood of some of the subjects was carefully studied. Catalase and glutathione peroxidase were defined for the assessment of the antioxidant system. Indicators of lipoperoxidation and antioxidant systems were studied during the experiment (before and after treatment).

Table 6 shows the intensity of the lipoperoxidation and antioxidation processes amongst people with toxic hepatitis, who take Cytaphat in comparison to Essentiale (average ± standard deviation of the average).

Acute exogenous poisoning and hepatitis, as a complication, cause a strong activation of the lipoperoxidation system and oppression of the

Table 5. The comparative description of the level of cytolysis ferments amongst patients with hepatitis 3 days after treatment.

Groups	Biochemical indicators of cytolysis		
	ALT nmol/(sl)	AST nmol/(sl)	Bilirubin mcmol/liter
Placebo	354.7 ± 45.0	297.1 ± 33.6	34.5 ± 3.7
Essentiale	232.2 ± 20.8	154.7 ± 19.6	24.6 ± 2.4
p	< 0.05	< 0.05	< 0.05
Cytaphat	248.9 ± 35.8	109.1 ± 8.7	19.8 ± 1.7
p	< 0.05	< 0.05	< 0.05
p	> 0.05	> 0.05	> 0.05

Table 6. Indicators of lipoperoxidation and antioxidation among patients using Essentiale and Cytaphat (average ± standard deviation of the average).

Indicators	Desirable (n=25)	Before treatment		After treatment	
		Essentiale (n=24)	Cytaphat (n=50)	Essentiale (n=24)	Cytaphat (n=50)
Malondialdehyde (erythrocytes mcmol/ml)	6.4 ± 0.1	7.4 ± 0.57	10.6 ± 1.2*	7.5 ± 0.79	10.7 ± 1.6*
Conjugated dienes (plasma nmol/(sl))	0.71 ± 0.05	3.55 ± 0.78*	3.1 ± 0.55*	1.3 ± 0.42*	2.3 ± 0.5*
Conjugated dienes (erythrocytes conventional unit)	32.2 ± 2.8	136.7 ± 16.5	110.0 ± 10.7*	96.6 ± 7.3*	86.4 ± 9.9*
Ketodienes (conventional units)	7.0 ± 0.2	166.4 ± 27.9	135.4 ± 15.4*	112.6 ± 17.2	138.6 ± 27.1*
SPP (conventional units)	0.49 ± 0.034	0.87 ± 0.1	0.8 ± 0.06*	0.9 ± 0.18	0.73 ± 0.08*
SSP (conventional units)	0.122 ± 0.016	0.4 ± 0.04	0.42 ± 0.05*	0.46 ± 0.052	0.35 ± 0.05*
Schiffbase (conventional units)	0.069 ± 0.0023	0.27 ± 0.023	0.318 ± 0.05*	0.25 ± 0.014	0.118 ± 0.08*
Glutathione peroxidase (units/ml per min)	157.9 ± 6.4	126.6 ± 8.9	137.7 ± 5.8*	152.1 ± 10.2*	152.5 ± 9.5*
Catalasenmol (H_2O_2/ml per min)	0.5 ± 0.09	0.28 ± 0.03	0.31 ± 0.037	0.39 ± 0.04*	0.30 ± 0.06

Note.
* p<0.05

activity of antioxidation enzymes (catalase and glutathione peroxidase). The activation of the lipoperoxidation system can be seen by the increase in the blood of almost all products of the mentioned system, that appear at different stages of the hyperlipoperoxidation process.

Three-day application of Cytaphat leads to positive dynamics in the oxidative homeostasis. The levels of SPP, SSP and Schiff base in plasma decrease. The activity of glutathione peroxidase rises, and this is of particular interest, because glutathione peroxidase is an enzyme that belongs to the system of exchange of glutathione, the major reserves of which are located in the liver.

For comparison, we can consider the changes of the same parameters using Essentiale.

Essentiale acts on the lipoperoxidation and antioxidation. In particular, it reduces the level of metabolites of the lipoperoxidation system – conjugated dienes in plasma and red blood cells, ketodienes in red blood cells – but it has almost no effect on the indicators of SPP, SSP and final products (Schiff base). Essentiale increases the activity of enzymes of antioxidation, almost normalizing the activity of glutathione peroxidase.

Complex and taking into account all the markers, analysis shows that the antioxidant effect of Cytaphat is comparable with the effect of such a well-known antioxidant and structural hepatoprotector as Essentiale.

The average period of elimination of toxic hepatopathy using Cytaphat is 2.8 days, Essentiale - 7.2 days, and a placebo - 10.6 days. The new drug Cytaphat has a strong hepatoprotective effect.

The tolerability was good, except for a single case in which the drug was withdrawn after the first injection because tachycardia (up to 120 bpm) evolved after injection and remained for longer than 30 min (1 ml of 0.01% propranolol was infused intravenously).

The efficiency of the drug is considered to be very high, normalization of the state took less time in comparison with standard treatment and a significant improvement was achieved immediately after the first injection.

Preliminary results give us a hope that Cytaphat can become a first domestic cytoprotector with high efficiency.

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