EFFECTIVENESS OF A NEW HERBAL AGENT IN THE CONDITIONS OF EXPERIMENTAL MEDICINAL HEPATITIS

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S u m m a ry. The objective: to study pharmacological activity of new complex plant agent granules "Polyherbagastrin" (intragastrically 900 mg/kg once daily) for hepatitis model induced by intragastric administration of aqueous suspension of tuberculostatics mixture (isoniazid 50 mg/kg, rifampicin 500 mg/kg, pyrazinamide 1500 mg/kg) within 14 days compared with the reference preparation containing extracts of Fumaria officinalis and Silybum marianum fruits (EFS) (intragastrically 35 mg/kg once per day). Hepatoprotective effect of new complex plant agent granules "Polyherbagastrin" was found. Normalizing effect of "Polyherbagastrin" on disorders of lipid levels in the blood serum, pro- and antioxidant balance was higher than the action of reference drug containing extracts of Fumaria officinalis and Silybum marianum fruits. Available pronounced hepatoprotective action substantiates the reasonability of further administration of the drug "Polyherbagastrin" to correct hepatotoxic action of anti-tuberculous agents.

K e y w o r d s: experimental hepatitis, herbal remedies, hepatoprotective activity.

INTRODUCTION

The treatment of patients with tuberculosis (TB) assumes the use of combined chemotherapy (4-6 drugs indicated simultaneously). It increases metabolic load on the liver – the main organ detoxicating xenobiotics. Due to this fact one of the first positions among complications of combined chemotherapy administered for TB patients is occupied by medicinal lesions of the liver. Their frequency is 60% against the ground of administration of the first course anti-tuberculous drugs, and it is 42,4% with administration of the second course drugs [6, 7, 8, 10]. Medicinal liver lesions prevent implementation of a full value etiotropic therapy and require its cancel in 11-28% of TB patients [3, 10]. Administration of hepatoprotectors is one of the main approaches to prevention and treatment of hepatotoxic reactions to antituberculous drugs [4]. Considering the mentioned above even at the preclinical examination of new potential hepatoprotectors investigation of their action on the experimental models of liver lesions caused by antituberculous drugs is rather topical.

THE AIM OF THIS STUDY

Objective: to assess the efficacy of a new complex herbal agent (CHA) in the form of granules under the code name "Polyherbagastrin" under conditions of experimental hepatitis caused by tuberculostatic agents.

MATERIALS AND METHODS

The CHA granules "Polyherbagastrin" and a comparison drug containing the extracts of common fumitory (*Fumaria officinalis*) and holy thistle (*Silybum marianum*) fruit were the objects of the study [4]. The granules "Polyherbagastrin" is a new complex pharmacological agent containing a native powder of sandy everlasting (*Helichrysum arenarium*) inflorescences, corn silk, herbs of horsetail (*Equisetum arvense*) and way-grass (*Polygonum aviculare*), horse chestnut (*Castanea*) fruit, roots of licorice (*Glycyrrhiza*) and wheat (*Triticum*) brans [2].

The experiment was performed on 40 albino outbred rats with the body weight of 190-210 g, 10 animals in each group. The animals were divided

into four groups: the 1st one – intact control, the 2nd group – untreated control, the 3rd group – animals with administered comparison drug, the 4th group – animals administered to the examined drug "Polyherbagastrin". In order to simulate hepatitis the mixture of tuberculostatic agents was introduced intragastrically to the rats from the second, third and fourth groups (isoniazid 50 mg/kg, rifampicin 500 mg/kg and pyrazinamide 1500 mg/kg) in the form of water suspension during 14 days [10]. The investigated agent granules "Polyherbagastrin" in the dose of 900 mg/kg and the comparison drug containing herbal extracts in the dose of 88 mg/kg were administered intragastrically a week before introducing the mixture of tuberculostatic agents and 1 hour before every day during 14 days. The applied dose (900 mg/kg) of the granules "Polyherbagastrin" was substantiated in the previous studies as the most effective [2]. The dose of the drug containing herbal extracts (88 mg/kg) was determined by means of calculation from the effective human dose using the species susceptibility coefficient [5].

The efficacy of the agent examined was determined by the following parameters: the percentage of animals survival, mass liver coefficient (MLC) [9], alanine aminotransferase (ALT) activity (Reitman-Frankel method by means of test-set "Lachema", produced by Czech Republic), the level of alkali phosphatase (ALP), concentration of cholesterol and lipids in the blood serum [9]; the content of thiobarbituric acid active products (TBA-AP), the level of reduced glutathione (GSH) in the liver homogenate and catalase activity in the blood serum [9]. The results were processed statistically by means of variation statistics methods with the applied program «STATISTICA® for Windows 6,0» (StatSoft Inc., USA) using Student criterion Bonferroni's amendment with statistical significance $p<0,05$ [1]. The experiment was performed according to the Regulations issued by the National Institute of Health and European Council Directives concerning care and use of laboratory animals (86/609/EEC), and approved by the local ethics committee.

RESULTS

The results of the experiment obtained are presented in the Table 1. Hepatitis caused by tuberculostatic agents was characterized by reliable changes of functional-biochemical indices in the animals from the untreated control group. 75% animal survival was registered, reliable increase of MLC concerning intact control group (4,02±0,13 against 3,50±0,15), an increased content of alkali phosphatase in the blood serum (10,27±0,51 against 7,22±1,09) and intracellular enzyme ALT (0,78±0,02 against 0,38±0,02). A reliable increase of cholesterol and general lipids levels concerning intact control in the blood serum was found: 2,41±0,22 against 1,31±0,10 g/L and 1,90±0,09 against 0,77±0,07 g/L respectively. A reliable increase of TBA-AP was found (0,97±0,03 against 0,74±0,02 mcmol/g) against the ground of decreased level of RG (2,20±0,10 against 3,27±0,08 mcmol/g) and antioxidant catalase enzyme (28,54±1,56 against 36,97±2,78 standard unit) (Table 1).

Table 1. Effect of "Polyherbagastrin" on thee indices characterizing liver functional disorders in rats in response to simulated hepatitis caused by tuberculostatic agents

Indices	Groups of animals			
	Intact control (n=5)	Untreated control (n=7)	Pathology + Granules "Polyherbagastrin", 900 mg/kg (n=6)	Pathology + CD HED, 88 mg/kg (n=6)
Survival, %	100	75	83	83
MLC, %	3,50±0,15	4,02±0,13 1	3,62±0,26 2	3,61±0,13 2
Blood serum				
ALT, mccat/L • hour	0,38±0,02	0,78±0,02 1	0,52±0,02	0,55±0,06 1/2
ALP, mcmol/sec • L	7,22±1,09	10,27±0,51 1	6,52±0,70 2	5,53±0,61 2
Cholesterol, g/L	0,77±0,07	1,90±0,09 1	0,73±0,09 2	0,78±0,06 2
General lipids, g/L	1,31±0,10	2,41±0,22 1	1,65±0,15 2	1,89±0,24
Catalase, standard units	36,97±2,78	28,54±1,56 1	50,32±0,16 2	54,60±9,08 1
Liver homogenate				
TBA-AP, mcmol/g	0,74±0,02	0,97±0,03 1	0,65±0,03 2	0,50±0,03 1/2
GSH, mcmol/g	3,27±0,08	2,20±0,10 1	3,13±0,16 2/3	2,65±0,09 1

Notes: 1) 1 - statistically reliable differences concerning intact control, $p<0,05$; 2) 2 - statistically reliable differences concerning untreated control, $p<0,05$; 3) 3 - statistically reliable differences concerning the comparison drug containing herbal extracts, $p<0,05$.

Under the influence of the granules "Polyherbagastrin" and the comparative drug a reli-

able decrease of MLC index concerning untreated control group was found (3,62±0,26 against 4,02±0,13% and 3,61±0,13 against 4,02±0,13% respectively) against the ground of 83% animals survival (Table 1), 1,5 and 1,4 times reduction of the marker cytolysis enzyme ALT respectively and a reliable 1,57 and 1,4 times decrease of alkali phosphatase respectively. The examined medicine "Polyherbagastrin" promoted reduction of both indices of lipid metabolism of general lipids and cholesterol in 1,46 and 2,6 times respectively. The comparison drug containing herbal extracts decreased only increased cholesterol level significantly (0,78±0,06 against 1,90±0,09 g/L) and did not affect the level of general lipids.

The granules "Polyherbagastrin" decreased statistically significant level of TBA-AP concerning untreated control (0,65±0,03 against 0,97±0,03 mcmol/g) and increased RG level (3,13±0,16 against 2,20±0,10 mcmol/g) and catalase activity in the blood serum (50,32±0,16 against 28,54±1,56 standard units). The comparison drug containing herbal extracts reliably decreased the level of TBA-AP (0,50±0,03 against 0,97±0,03), although it did not increase GSH level concerning untreated control group (2,65±0,09 against 2,20±0,10 respectively), and an increased activity of the antioxidant enzyme catalase was unreliable (54,60±9,08 against 28,54±1,56 standard units, $p>0,05$).

DISCUSSION

Isoniazid and rifampicin are known to be inductors of the cytochrome isoforms CYP 2E1 and 3A4 [7, 10]. CYP3A4 activation results in increased metabolism of isoniazid with formation of toxic metabolites, which explains potentiation of hepatotoxic effect in case of simultaneous introduction of these drugs. Rifampicin stimulates isoniazid-hydrolase resulting in increased formation of hydrazine, especially in patients with slow acetylation and therefore increasing toxic potential of the drugs [7]. Pyrazinamide is able to activate lipid peroxide oxidation (LPO) exhausting antioxidant reserves, damaging membrane structures of blood cells, potentiating hepatotoxic effect of rifampicin and isoniazid [10]. The described disorders were evidenced by the results of our studies. Experimental hepatitis caused by tuberculostatic agents was characterized by a reliable increase of MLC concerning intact control group, which is indicative of development of infiltration-inflammation processes in the organ. It is evidenced by reliable increase of alkali phosphatase. Due to intensified cytolysis, as a result of membrane structures degradation, a reliable increase of the intracellular enzyme ALT in the blood serum occurred. Due to disorders of the liver metabolic function a reliable increase of cholesterol level and general lipids in the blood serum occurred concerning intact control group. Disorders in the pro- and antioxidant balance are evidences of pathological processes in the organ: increased processes of LPO and decreased activity of antioxidant protection were found. A reliable increase of TBA-AP was found against the ground of reduced level of RG and antioxidant catalase enzyme. It is indicative of intensification of LPO processes and functional failure of glutathione and enzymatic protection of the organ.

Under the effect of the granules "Polyherbagastrin" in the dose of 900 mg/kg, and the drugs containing herbal extracts effect as well reduction of infiltration-inflammatory processes in the organ were found, which is evidenced by a reliable decrease of MLC index concerning untreated control group against the ground of 83% of animals survival (Table 1). A positive effect of granules "Polyherbagastrin" on cytolytic processes in the liver is manifested in a reliable decrease of the marker cytolysis enzyme ALT concerning untreated control group. These results are indirectly indicative of restoration of the blood cells integrity in case of administration of granules "Polyherbagastrin" in the dose of 900 mg/kg on the level of the comparison drug.

Contrary to the comparison drug reliably reducing only increased level of cholesterol and did not affect the level of general lipids, the granules "Polyherbagastrin" promoted reduction of both indices if lipid metabolism. It is indicative of more effective normalizing effect of the examined agent "Polyherbagastrin" on the liver function.

Granules "Polyherbagastrin" manifested pronounced antioxidant properties, statistically significant decreasing the level of TBA-AP, increasing the level of GSH and catalase activity in the blood serum concerning untreated group. Concerning the degree of a normalizing effect on the disturbed pro- and antioxidant balance the agent "Polyherbagastrin" was higher than that of the comparison drug.

Hepatoprotective action of the comparison drug is known to be insured by the flavonoids of common fumitory (*Fumaria officinalis*) and holy thistle (*Silybum marianum*) fruit. Advantages of the examined drug over the drug of comparison might be explained by more variable content of plant fla-

vonoids providing more powerful antioxidant and membrane-stabilizing action, as well as hypolipidemic properties of wheat brans [11].

Therefore, the new agent "Polyherbagastrin" manifests hepatoprotective effect on stimulated hepatitis model caused by anti-tuberculous agents. By the effect of normalizing action on disorders of lipid level in the blood serum, pro- and antioxidant balance, the drug "Polyherbagastrin" prevails the action of the reference-drug containing the extracts of common fumitory (*Fumaria officinalis*) and holy thistle (*Silybum marianum*) fruit.

CONCLUSIONS

1. Hepatoprotector effect of the plant agent "Polyherbagastrin" on simulated hepatitis model caused by anti-tuberculous agents is determined.
2. By the effect of normalizing action on disorders of lipid level in the blood serum, pro- and antioxidant balance, the drug "Polyherbagastrin" prevails the action of the reference-drug containing the extracts of common fumitory (*Fumaria officinalis*) and holy thistle (*Silybum marianum*) fruit.
3. Available pronounced hepatoprotective action substantiates the reasonability of further administration of the drug "Polyherbagastrin" to correct hepatotoxic action of anti-tuberculous agents.

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