The results show that EOS plays a direct part in the formation of the response of changes in BP and HR to presentation of emotionally meaningful stimuli to lower primates. Nalaxone, when injected intravenously, depending on its dose may have opposite actions on the intensity of the responses of changes in BP, for it acts primarily on changes in BP developing in response to CS. This is evidently connected with its interaction, in a dose of 0.1 mg/kg, predominantly with μ-opiate receptors, and only if the dose is increased does it block δ-receptors, with which the hypertensive action of opioids is associated [1]. Attention must also be drawn to the similarity of action of morphine and naloxone in a dose of 1 mg/kg. In the writers' opinion, the reason for this is that morphine, which is an agonist of μ-receptors, may behave as an antagonist relative to endogenous substances that interact with δ-receptors.

The investigation also showed that injection of naloxone into PVH considerably facilitates the response of the CVS to emotiogenic stimuli, whereas its injection into NTS completely blocks the rise of BP in this situation. We know that PVH, which is rich in opiatergic structures [3, 4], is a key region in the formation of the autonomic response of lower primates to emotionally meaningful stimuli [5]. It can thus be concluded that the opiatergic structures of PVH and NTS participate actively in the formation of the response of BP to emotionally meaningful stimuli. Incidentally, if the effects of suppression of the responses of BP and HR to CS and US can be completely transmitted through PVH and NTS, the points of application of the effect of naloxone in doses acting chiefly on μ-receptors, and also probably of morphine, may be other brain structures or structures of the autonomic nervous system.

LITERATURE CITED

1. E. V. Golanov, The Present State of the Problem of Endogenous Morphine-like Substances [in Russian] Moscow (1986).
2. G. Feuerstein, R. L. Zerbe, and A. J. Faden, Hypertension, 5, 663 (1983).
3. T. Hokfelt, R. Elde, O. Johansson, et al., Neurosci. Lett., 5, 25 (1977).
4. T. Hokfelt, L. Terenius, H. G P. J. Kuypers, and R. Daum, Neurosci. Lett., 14, 55 (1979).
5. O. A. Smith, C. A. Astley, J. L. De Vito, et al., Fed. Proc., 39, 2487 (1980).
6. S. Szekely, Opioid Peptides, Vol. 2 New York (1982), pp. 109-154.
7. R. Rhone, Atlas Stereotaxique du Cerveau du *Papio hamadryas*, Paris (1976).

EFFECT OF RATIBOL, RETABOLIL, AND SOLASODIN ON THE BLOOD CLOTTING SYSTEM

R. D. Seifulla, M. A. Vaisberg, S. Ya. Sokolov, E. K. Kim, and Kh. D. Bairamkulov

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KEY WORDS: ratibol; blood coagulation; hemostasis.

This paper describes a study of the effect of three preparations, retabolil, ratibol, and solasodin, on the blood clotting system and on fibrinolysis; the first of the three is widely used in clinical practice in various diseases, and the last two have been approved by the Pharmacological Committee of the USSR for clinical trials.

Since one side effect of retabolil is its damaging action on the liver, especially if used over a long period of time, a closer study of the behavior and interconnection between blood coagulation factors, synthesized in the liver in response to repeated injections of retabolil, would appear to be an urgent task.

Department of Physicochemical and Biological Control Methods, All-Union Research Institute of Physical Culture, Moscow.

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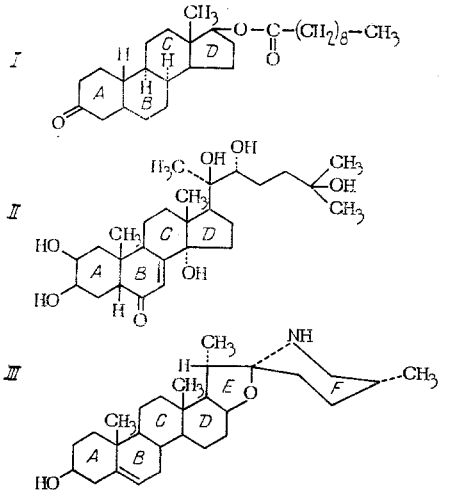

Fig. 1. Structural formulas of retabolil (I), ratibol (II), and solasodin (III).

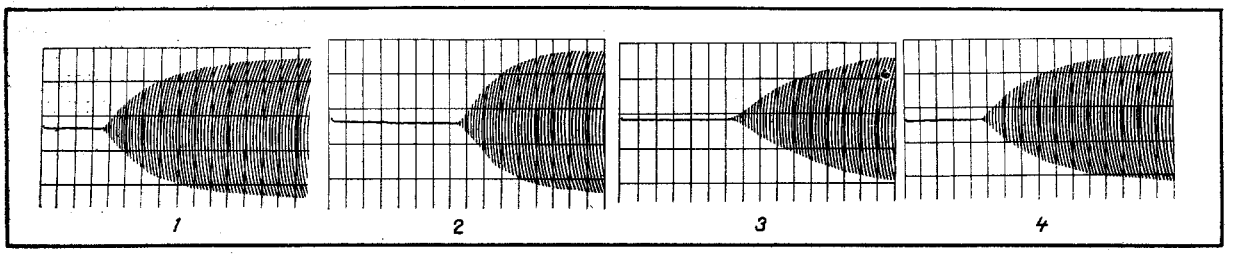

Fig. 2. Thromboelastograms of rabbit blood. 1) Normal; 2, 3, 4) 24 h after peroral administration of a single dose of retabolil, ratibol, and solasodin, respectively.

Ratibol is an anabolic preparation of plant origin isolated from the root of *Leuzea carthamoides* (synonym: *Rhapontieum carthamoides*), or rhapontic rhubarb. Indications for the use of ratibol are currently being worked out. It is less active as an anabolitic agent than retabolil, and is characterized more by its qualities of an adaptogen of plant origin. There is no information about the effect of ratibol on blood coagulation and hemostasis.

In several of its pharmacological properties solasodin resembles the first two preparations, but also has differences.

All three preparations possess a steroid structure, for they are all derivatives of cyclopentanephenanthrene. Solasodin has additional E and F rings (Fig. 1).

EXPERIMENTAL METHOD

The mechanisms of action of retabolil, ratibol, and solasodin on the blood clotting system were investigated by thromboelastography and by determination of the blood heparin tolerance, activity of the prothrombin complex by Quick's test, activity of factors II (prothrombin), V (Ac-globulin [11]), VII (proconvertin [10]), XIII (fibrin-stabilizing factor [12]), and I (fibrinogen) and also activity of fibrinolytic enzymes, determined by two methods [6, 10]. Tolerance of the fibrin clot to streptokinase and antithrombin activity also were investigated.

The steroid compounds for testing were used in doses of 1-5 and 10 mg/kg in the case of a single dose and 1 mg/kg for administration over a period of 20 days. When a single dose of retabolil, ratibol, and solasodin was given the investigations were carried out in the initial state and after 2, 6, 12, 24, 48, and 72 h. During repeated peroral administration of the preparations samples were taken in the initial state on the 5th, 10th, 15th, and 20th days, and also 3, 10, and 15 days after discontinuation of the preparations. Tests were carried out on 214 rabbits of both sexes. Series of experiments also were undertaken in vitro, in which the preparations were added to blood or plasma 30 min before investigation in concentrations of 10 to 1090 μg/ml, and incubated. The results were subjected to statistical analysis [1].

TABLE 1. Effect of Retabolil, Ratibol, and Solasodin, Administered in a Daily Dose of 1 mg/kg for 20 days, on Activity of Prothrombin Complex in Quick's Test

Period of investigation	Number of animals	Retabolil	Ratibol	Solasodin
Initial state (control)	18	100,5±8,7	98,4±10,3	107,5±11,5
Administration (days)				
5-th	18	72,4±6,4*	78,1±6,3*	100,4±9,2
10-th	18	66,5±4,9*	71,5±6,3*	96,4±7,2
15-th	18	63,3±7,8*	91,7±10,3	70,4±5,2*
20-th	18	57,2±6,3*	145,8±10,3*	65,1±8,5*
After administration (days)				
3-rd	18	55,8±4,9*	100,4±8,1	110,9±12,5
10-th	18	69,9±6,1*	106,3±9,2	100,5±10,3
15-th	18	106,8±8,3	96,4±9,9	102,8±8,3

Legend. Here and in Table 2: *p < 0.05 compared with control; six animals were tested with each preparation.

TABLE 2. Effect of Retabolil, Ratibol, and Soladosin in a Dose of 1 mg/kg Daily for 20 days on Fibrinolytic Activity (in %)

Period of investigation	Number of animals	Retabolil	Ratibol	Solasodin
Initial state	18	19,7±2,1 100,4±9,5	20,5±1,5 108,5±12,3	18,9±2,1 105,3±9,2
Administration (days)				
5-th	18	18,7±1,3 98,3±11,4	19,9±2,1 99,4±8,3	20,5±2,4 101,2±5,3
10-th	18	32,5±4,1* 141,7±15,2*	30,2±2,5* 139,9±12,1*	18,9±1,4 105,4±12,5
15-th	18	38,2±5,1* 155,7±14,7*	35,3±2,4* 142,8±12,3*	29,9±1,2* 138,9±11,6*
20-th	18	43,7±3,5* 159,7±18,2*	22,4±4,8 105,4±9,5	37,1±2,9* 141,3±15,7*
After administration (days)				
3-rd	18	33,4±2,1* 145,7±17,3*	19,2±1,5 100,6±5,8	21,3±1,7 102,3±8,8
10-th	18	18,5±1,8 100,4±20,7	19,9±2,5 107,2±9,2	15,8±1,9 100,5±17,4
15th	18	19,1±1,2 107,4±12,8	20,5±1,7 100,3±10,2	18,9±1,8 99,4±10,8

Legend. Above the line: fibrinolysis determined by Bidwell's method; below the line: fibrinolysis determined by Mitchell's method.

EXPERIMENTAL RESULTS

After peroral administration of a single dose of 5-10 mg/kg of retabolil, ratibol, and solasodin to the rabbits lengthening of the time intervals characterizing the reaction time r and the time of beginning of clot formation K was observed, depending on the dose of the preparation (Fig. 2). Activity of the prothrombin complex in Quick's test and heparin tolerance were reduced. Ratibol had a biphasic effect on blood coagulation depending on the duration of its administration, as was confirmed by changes in the prothrombin complex and blood heparin tolerance (Table 1). In the first phase there was a decrease, in the second phase an increase, in the coagulation potential of the blood. The possibility cannot be ruled out that this was connected with its transformation in vivo into other steroid compounds which may have an opposite action on blood coagulation.

With respect to retabolil the results obtained agree in principle with those found by other investigators, who demonstrated inhibition of platelet adhesion and aggregation by the preparation [4]. Savel'eva [5] considers that the antiadhesive and antiaggregation actions of retabolil are connected with intensification of synthesis of phospholipids which inhibit the above-mentioned functions of the platelets, and with the release of biologically active metabolites, including ADP, from platelet membranes.

We concluded from the results that retabolil acts primarily on the first phase of blood coagulation (the formation of active thromboplastin) and to a lesser degree on the second stage (thrombin formation).

Data on the action of solasodin on the kinetics of blood coagulation are additional to results published previously.

In a study of the action of retabolil, ratibol, and solasodin on blood coagulation factors all three preparations were found to reduce activity of factors II and VII.

An important feature distinguishing the action of retabolil, ratibol, and solasodin is a reduction of the blood factor I concentration in rabbits after a single dose of the preparations and after their administration for 20 days. As regards both doses and times of investigation, these data agree with the reduction of activity of factor XIII (fibrin-stabilizing factor) discovered previously. The steroid preparations under investigation not only reduce the quantity of fibrinogen, the substrate for blood coagulation, but they also reduce the possibility of conversion of fibrin S into fibrin i. In a clinical study retabolil, given to patients with ischemic heart disease, reduced the fibrinogen concentration and fibrinase activity [2].

Retabolil, ratibol, and solasodin increased the fibrinolytic activity of the experimental animals by different degrees. The effects found are characteristic of doses of 5 and 10 mg/kg in the case of a single dose and 1 mg/kg in the case of a 20-day course (Table 2).

These results for retabolil are in agreement with data in the literature according to which this anabolic steroid, under clinical conditions, averts the risk factor and reduces the fibrinolytic potential [2]. This effect is evidently connected with release of tissue proactivator and urokinase, and also with other causes.

The experiments in vitro also showed that retabolil, ratibol, and solasodin reduce the resistance of the fibrin clot to streptokinase. For instance, administration of these preparations to animals in single doses of 5 or 10 mg/kg at the corresponding time or in a dose of 1 mg/kg daily for 20 days lead to induced lysis of the fibrin clot by the standard preparation, activating profibrinolysin by streptokinase.

The study of the effect of retabolil, ratibol, and solasodin on the blood antithrombin activity of the animals showed that the thrombin time is lengthened both after a single large dose and after a 20-day course.

Thus retabolil, ratibol, and solasodin possess moderate anticoagulant activity. The mechanism of their action consists of inhibition of formation of active thromboplastin and thrombin, inhibition of fibrin formation from fibrinogen (especially the conversion of fibrin S into fibrin i), and activation of fibrinolysis. This is connected with their effect on activity of the most important coagulation factors: II, I, and XIII.

These steroids have no effect on factor V. The same pattern also was found with respect to their action on fibrinolytic and antithrombin activity of the blood. In all probability this effect of retabolil, ratibol, and solasodin is linked with their action on metabolism. These compounds have no direct effect in vitro. For a final explanation of these problems further investigations of the molecular mechanisms of synthesis and activation of blood coagulation factors are necessary.

LITERATURE CITED

1. M. L. Belen'kii, Elements of Quantitative Evaluation of a Pharmacological Effect [in Russian], 2nd edition, Leningrad (1963).
2. A. T. Karacharov, Klin. Med., No. 10, 131 (1971).
3. K. M. Lakinand R. D. Seifulla, Probl. Endokrinol., No. 4, 97 (1966).
4. K. M. Lakin, V. A. Makarov, N. V. Novikova, and V. M. Tret'yak, Farmakol. Toksikol., No. 3, 113 (1983).
5. A. V. Savel'eva, Farmakol. Toksikol., No. 1, 62 (1973).
6. E. Bidwell, Biochem. J., 55, 497 (1953).
7. P. Jarett and I. Scurr, Int. J. Haemostas. Thrombos. Res., 7, Suppl. 1, 57 (1982).
8. J. Jürgens and F. K. Beller, Klinische Methoden der Blutderinnungs Analyse, Stuttgart (1959).
9. F. Koller, A. Loeliger, and F. Ducret, Acta Haematol., 6, 1 (1951).
10. I. Mitchell, cited by E. Shcheklik, Clinical Enzymology [Russian translation], Warsaw and Moscow (1966), pp. 197-205.

11. P. Owrén, Biochem. J., 43, 136 (1948).
12. P. Sigg and F. Duckert, Schweiz. Med. Wschr., 93, 1455 (1963).
13. M. Small, J. J. Douglas, J. O. Lowe, and C. D. Forbes, Lancet, 1, 1114 (1983).

EFFECT OF HEPATOPROTECTORS ON LIPID METABOLISM IN HEPATITIS INDUCED BY CARBON TETRACHLORIDE

A. I. Vengerovskii, V. S. Chuchalin, O. V. Paul's, and A. S. Saratikov

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KEY WORDS: silybinin, essentiale, lipids, carbon tetrachloride, hepatitis.

An important role in the pathogenesis of toxic hepatitis is played by the detergent action of lysophospholipids and activation of phospholipases and of lipid peroxidation (LPO), leading to the development of cytolysis of the hepatic parenchyma [2, 3]. The mechanism of the hepatoprotective effect of phospholipid preparations and antioxidants of phenolic nature is considered to be through their inhibitory effect on individual components of the "lipid triad" [5, 11, 13].

The aim of this investigation was to study the effect of the widely used hepatoprotective agents — the flavonoid silybinin and the phosphatidylcholine-containing substance essentiale — on the combination of disturbances of lipid metabolism present in severe toxic hepatitis induced by carbon tetrachloride (CCl_4).

EXPERIMENTAL METHOD

Experiments were carried out on 180 male albino rats weighing 200-220 g. For 4 days the animals were given 2.5 ml/kg of a 50% solution of CCl_4 in olive oil by gastric tube daily, accompanied by an aqueous suspension of silybinin (200 mg/kg; Karsil, from Bulgaria) or a solution of essentiale (80 mg/kg; from Yugoslavia) in ampuls. The doses of the hepatoprotective agents were chosen beforehand in a screening experiment. Control animals received CCl_4 and, instead of the hepatoprotective agents, the same volume of distilled water. The concentrations of total lipid and phospholipid fractions in the lipid extracts of the liver [7] were determined by one-way thin-layer chromatography on Silufol UF-254 plates (Czechoslovakia) [1], and the concentrations of diene conjugates (DC) [3] and Schiff bases [14], and the antiradical activity of lipids [10] also were determined. The reduced glutathione concentration [12] and the kinetics of malonic dialdehyde (MDA) formation were studied in liver homogenates perfused with KCl solution and Tris-buffer (pH 7.4), during stimulation of LPO in vitro by Fe^{++} and ascorbic acid, or by an enzymic method [3]. Activity of acid phosphatase and β-hydroxybutyrate dehydrogenase (HBDH) was determined histochemically in frozen sections of the liver, followed by cytophotometry. The number of necrotic hepatocytes was counted in survey sections through the liver stained with hematoxylin and eosin. Phospholipase A activity [6] and concentrations of total lipids, phospholipids, and low-density and very low-density lipoproteins [4] were measured in the blood serum.

EXPERIMENTAL RESULTS

CCl_4 caused a profound disturbance of lipid metabolism. On the 4th day of poisoning the lipid concentration in liver homogenates was increased by 2.4 times, mainly due to an increase (by 5.4 times) in the amount of triglycerides (Table 1). The concentration of free cholesterol and of its esters also was increased. The relative percentage of triglycerides was increased by 2.3 times, the level of free cholesterol was not significantly changed, and concentrations of monodiacylglycerides, free fatty acids, and cholesterol esters were reduced by 1.7-1.9 times.

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