PHARMACOLOGY AND TOXICOLOGY

Experimental Study of Biological Activity of Angiogen in Experimental Cardiovascular and Hemostasis Pathology

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Positive effects of angiogen, a preparation containing succinic and acetylsalicylic acids, on ECG, blood clotting, and lipid metabolism were demonstrated on animals with experimental cardiovascular disease induced by repeated injections of norepinephrine.

Key Words: *angiogen; cardiovascular disease; therapy*

Pathology of the cardiovascular system (CVS) reduces quality of life, often leads to disability and early death. Despite a wide armory of therapies for these diseases, intensive search for new more effective drugs for their treatment is in progress.

An actual trend is the search for new drugs normalizing myocardial metabolism and preventing thrombosis and embolism often accompanying cardiovascular pathology [1,4].

The aim of the present study was to evaluate the efficiency of angiogen, a new Russian-made drug containing succinic acid improving myocardial metabolism [2,4] and acetylsalicylic acid, an effective antiaggregant [6,11]. We elucidated whether these substances retained their activity in the complex preparation.

Cardiovascular pathology was modeled by intraperitoneal injection of norepinephrine (NE) inducing disorders in cardiac activity, blood clotting system, and lipid metabolism, similar to those observed in cardiovascular patients [7].

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MATERIALS AND METHODS

Experiments were carried out on male albino rats (150-180 g) from Rappolovo Breeding Center. Group 1 animals received angiogen in a therapeutic dose of 50 mg/kg for 1 week through a gastric tube. Group 2 animals were intraperitoneally injected with NE according to the following protocol: 2 mg/kg on day 1, 3 mg/kg on day 2, and 4 mg/kg on days 3-7 [3]. After the end of NE treatment some of these rats were treated with angiogen (50 mg/kg) for 5 days and others received no treatment. Group 3 animals were injected with NE according to the above scheme simultaneously with angiogen in a dose of 50 mg/kg. Intact rats were controls.

Systolic blood pressure (SBP) was measured using an Ugo Basile blood pressure recorder. ECG was recorded and rectal temperature measured on an RM-6000 polygraph. To this end, the animals were put into special narrow cages. ECG was recorded using subcutaneous stainless steel electrodes. Bleeding time and complete blood clotting time were evaluated routinely [8]. Blood was collected from the caudal vein or from the decapitation wound.

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Serum lipids were measured by the Knight method, cholesterol was assayed as described previously [5]. Platelets were counted by the Fonio method [5], platelet adhesive capacity was measured by a qualitative macroscopic method in a tube with platelet-rich plasma [5].

The data were statistically processed using Student's *t* test at $p<0.05$.

RESULTS

Repeated injections of NE induced considerable disorders in cardiovascular activity: decrease in *P* and *R* wave amplitude, increase in S wave amplitude, lengthening of ventricular conduction time (*QT* interval), increase in systolic index, and decrease in SBP. In 70% animals *ST* depression and negative *T* waves were observed. These changes attested to serious circulatory and metabolic disorders in the myocardium and low contractile activity of the heart.

In animals receiving NE and angiogen simultaneously these changes in the cardiovascular system were less pronounced compared to animals treated with NE alone. Pathological changes in ECG were less numerous (*ST* depression and negative *T* wave).

Injection of NE induced disorders in platelet hemostasis: increase in platelet count and adhesion capacity, accelerated platelet aggregation. These changes attested to activation of the blood clotting system (Table 1). This was confirmed by a decrease in bleeding time and complete clotting time. Changes in the blood clotting system were paralleled by accumulation of total lipids and cholesterol due to their mobilization from lipid depots. Treatment with angiogen simultaneously with NE prevented the development of these pathological symptoms. Platelet adhesion capacity and serum content of total lipids virtually did not differ from normal.

Treatment with angiogen alone produced no appreciable changes in ECG and SBP, and induced only slight hypothermia (Table 1). A decrease in platelet count, deceleration of their aggregation and decreased adhesion capacity, prolongation of bleeding time and complete clotting time, and decreased level of cholesterol were observed, but these parameters remained within the normal range, although in some cases changes were statistically significant (Table 1).

After NE was discontinued, platelet hemostasis parameters and ECG did not returned to normal for 5 days. *ST* depression, negative *T* wave, decreased of *P*

TABLE 1. Effect of Angiogen on Cardiovascular Parameters, Serum Content of Total Lipids and Cholesterol, and Blood Clotting Parameters in Rats Treated with NE (*M*±*m*)

Parameter	Intact	Angiogen	NE	NE+angiogen
Heart rate, bpm	492±34	493±27	483±11	470±24
P, mV	0.16±0.02	0.14±0.01	0.075±0.005*	0.079±0.007*
R, mV	0.75±0.09	0.69±0.05	0.61±0.08	0.71±0.14
S, mV	0.13±0.10	0.11±0.04	0.23±0.03^{+}	0.086±0.05
T, mV	0.23±0.04	0.21±0.03	0.15±0.03	0.19±0.03
PQ, msec	42.0±0.9	44±1	44±1	43.0±1.5
QT, msec	58±2	63.0±1.5	71±2*$^{+}$	61±3
RR, msec	125±3	125±8	127±4	130±6
Systolic index, %	48±4	52±4	56±2^{+}	47.0±0.8
SBP, mm Hg	111±4118±7	95±3	100±3	
Pathological ECG, %	0	0	70*	29
Total serum lipids, g/liter	3.7±0.3	3.0±0.1*	4.5±0.1*	3.5±0.1
Serum cholesterol, mmol/liter	1.6±0.2	1.4±0.1	3.6±0.3*	2.3±0.2*
Temperature, °C	37.8±0.3	36.9±0.6	37.2±0.3*	38.3±0.2
Bleeding time, min	5.3±0.4	5.6±0.3	3.1±0.4*	3.9±0.3*
Complete clotting time, min	6.6±0.1	7.0±0.2	6.0±0.4	7.8±0.4*
Platelets, 10^9/liter	720±40	630±30	820±25*	750±20
Platelet adhesion capacity, %	35±5	26±5	60±10*	36±4
Time of platelet aggregation, sec	25±3	40±6*	10±2*	20±5

Note. $p<0.05$: **vs.* intact animals, *$^{+}$vs.* NE+angiogen group.

TABLE 2. Effect of Angiogen on Cardiovascular Parameters, Serum Content of Total Lipids and Cholesterol, and Blood Clotting Parameters in Rats (*M*±*m*)

Parameter	NE	NE+angiogen
Heart rate, bpm	463±67	428±12
P, mV	0.11±0.01*	0.10±0.02
R, mV	0.56±0.15	0.7±0.1
S, mV	0.31±0.17	0.15±0.05
T, mV	0.21±0.04	0.17±0.03
PQ, msec	45.0±5.6	43.0±1.8
QT, msec	81±15	67.0±1.8*
RR, msec	136±21	141±4*
Systolic index, %	60.0±5.9	48.0±2.3
SBP, mm Hg	108±11	109±10
Pathological ECG, %	50	0
Total serum lipids, g/liter	4.2±0.2+	3.3±0.2
Serum cholesterol, mmol/liter	3.4±0.1+	2.1±0.3
Temperature, °C	36.0±2.2	37.1±0.4
Bleeding time, min	3.6±1.4	4.2±0.4
Complete clotting time, min	6.3±0.6	7.6±1.2
Platelets, 10^9/liter	830±20+	740±30
Platelet adhesion capacity, %	60±8+	37±2
Time of platelet aggregation, sec	12±2+	20±4

Note. $p<0.05$: **vs.* intact animals (Table 1), *+vs.* treated animals.

and *R* wave amplitudes, increased *S* wave amplitude, marked lengthening of the *QT* interval and increase in systolic index were observed in 50% animals. Platelet hemostasis also did not return to normal after NE discontinuation (Table 2). Angiogen therapy after NE discontinuation normalized ECG. *ST* segment returned to isoline and *T* wave became positive (Table 2). Platelet hemostasis, serum concentrations of total lipids and cholesterol were almost normal (Table 2).

The effect of angiogen can be explained by optimization of metabolic processes in the myocardium via activation of succinate dehydrogenase oxidation and intensification of oxygen diffusion in the myocardium [4]. ECG parameters, for example elevation of *ST* segment, indicate normalization of blood supply and improved oxygen supply to the myocardium. Angiogen promoted recovery of platelet hemostasis disordered by NE, hence acetylsalicylic acid retained its capacity to inhibit platelet aggregation in this complex preparation. A decrease in plasma concentration of total lipids and cholesterol is practically significant.

Our findings suggest that both components of angiogen (acetylsalicylic and succinic acids) retain high biological activity in experimental pathologies of the cardiovascular and blood clotting systems.

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