EFFECT OF CONTROLLED CHANGES IN THE PHYSIOLOGICAL STATE OF CELL MEMBRANES ON THE COURSE OF A PATHOLOGICAL PROCESS

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UDC 616-08:616-018.18-085.7

Tissue homeostasis is largely connected with the normal functioning of the microvascular bed, and, in particular, with transcapillary interchange and penetration of physiologically active and other substances through the cell membranes of different tissues.

In pathological conditions this homeostasis may be disturbed in various ways. Because of this, controlled pharmacological action on the permeability of cell membranes, including the microvascular bed, is of considerable importance.

During the last few years experimental investigations have been carried out in this direction in the authors' laboratory using models of various pathological processes (radiation sickness, influenzal infection, metastasization of tumors, disturbance of the coronary circulation, and so on).

Considerable attention has recently been paid to the study of synthetic derivatives of vitamin P. The authors have studied the pharmacological properties of esculamine (8-dihydroxydiethylaminomethyl-4-methylesculetin hydrochloride), synthesized in the Institute of Pharmacology and Chemotherapy, Academy of Medical Sciences of the USSR [5], and have observed its normalizing effect on tissue and vascular permeability.

To judge the state of the tissue and vascular permeability, methods of studying the permeability of the tissue-blood barriers have been used, with sulfacyl-sodium [9] as an example of low molecular weight substances and fluorescein (production of a fluorescein-protein complex) as an example of substances completely bound (in a certain concentration) by blood proteins. The coefficient of permeability (K) was expressed by the ratio between the concentration (in mg%) of indicator in the organ and its concentration in the plasma.

EXPERIMENTAL METHOD AND RESULTS

In the first stage of the investigation the action of esculamine was studied on animals irradiated with x rays, for an essential role in the mechanism of the hemorrhagic syndrome arising after irradiation is played by changes in permeability of the tissue-blood and vascular barriers and the resistance of the vessels.

Animals (rats, rabbits, mice) were exposed to a single dose of whole-body irradiation from the RUM-3 x-ray apparatus, amounting to 600 or 800 R.

The sulfacyl-sodium concentration was determined by means of the FÉK-3M photoelectric colorimeter, and the fluorescein concentration by an electrofluorometric apparatus described by V. A. Gromov and Z. N. Nakhil'nitskaya [4], and also by L. B. Utevskaya's method [11]. The state of the vascular permeability was also judged from the amount of fluorescein (intensity of fluorescence in μA) escaping from the blood vessels into the aqueous humor of the anterior chamber of the eye and into the skin of rabbits. Esculamine was injected intravenously in a dose of 10 mg/kg or injected subcutaneously or given by mouth in a dose of 35-50 mg/kg (once or repeatedly).

The experimental results given in Table 1 show that in animals irradiated with x rays, esculamine lowers the tissue permeability, largely prevents the increase in permeability of the blood vessels of the blood-eye barrier and skin of rabbits, and restores normal vascular resistance, its activity exceeding that of Rutin. O. V. Alekseev [1] showed by means of the electron microscope that esculamine prevents marked dilatation of the interendothelial spaces in the capillaries observed after whole-body x-ray irradia-

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 64, No. 11, pp. 60-64, November, 1967. Original article submitted June 30, 1967.

TABLE 1. Effect of Esculamine and Rutin on Permeability and Resistance of Blood Vessels of Irradiated Animals (M ± m)

Group of animals	Dose of preparation (in mg/kg by mouth)	Number of animals	Resistance of vessels (number of petechiae)	Permeability				Length of survival	Number of animals
				tissue		vascular (μA)			
				coefficient of permeability of spleen	ratio to control	dose (in mg/kg subcutaneously)	blood-eye barrier / skin		
rats							rabbits		
Healthy	–	33	61 ± 6	0.32 ± 0.03	1	–	7.0 ± 0.07 / 9.7 ± 0.07	40	14
Irradiated, not receiving any preparations	–	43	157 ± 16	0.57 ± 0.03	1.77	–	40.1 ± 7.7 / 25.0 ± 5.5	20	14
Receiving rutin	50	10	114 ± 10	0.55 ± 0.08	1.72	182*	26.3 ± 9.7 / 15.2 ± 1.6	26	7
Receiving esculamine	50	14	40 ± 7	0.39 ± 0.05	1.22	35	17.4 ± 4.7 / 13.7 ± 2.6	33	7

*Urutin – a mixture of rutin, methenamine, and distilled water.

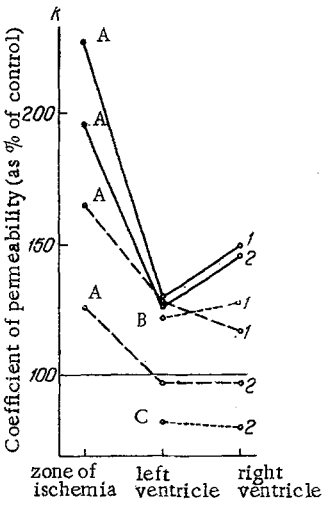

Fig. 1. Changes in permeability of coronary vessels and myocardium for fluorescein (continuous line) and sulfacyl-sodium (broken lines) 24 h after ligation of coronary artery (A) and irritation of vagus nerve (B). 1) Without preparation; 2) after injection of esculamine. Permeability in intact animals taken as 100.

tion of animals. Finally, the distinctive "strengthening" action of esculamine on cell membranes may also be judged by the lesser degree of penetration of ink particles both into spleen cells and into erythrocytes. This is confirmed also by the higher resistance of erythrocytes from irradiated animals receiving esculamine to alkaline hemolysis, indicating that the degree of injury to the erythrocyte membranes of animals irradiated with x rays is diminished by esculamine.

Esculamine thus has a normalizing action on tissue and vascular permeability when disturbed by x-ray irradiation.

In the second section of the investigation the effect of esculamine and aminoadamantan (the latter was synthesized by A. P. Arendaruk and co-workers [2]) on the course of experimental influenzal infection was studied. The basic assumption was that the state of vascular and cellular permeability is of great importance for penetration of microorganisms and viruses into the tissues and cells of the host. In addition, in clinical observations [15] an antiflammatory action of substances with vitamin P activity was observed, and their therapeutic effect has been attributed mainly to removal of the syndrome of capillary damage caused by bacteria or viruses.

Meanwhile substances capable of blocking the pathways of penetration of virus through the animal cell membrane – aminoadamantan and other amines – have aroused considerable interest [13, 14].

The effect of these preparations on the development and course of influenzal pneumonia were studied in experiments on albino mice infected intranasally under superficial

TABLE 2. Effect of Substances on Survival Period of Mice (in days)

Groups of animals	Dose of virus (in LD_{50})				
	1	2-3	5	10	50
Control	34	12.1	11.7	8.4*	5.8*
Receiving esculamine	53.1	35.8	23.7	12.2*	6.9*
Receiving aminoadamantan	52.3	41.0	29.0	25.0	7.8*

*Difference not statistically significant (P-0.05).

ether anesthesia with influenza A virus (strain PR-8) in doses of 1, 5, 10, and 50 LD_{50}. The substances were given internally to the animals in a dose of 100 mg/kg 10-30 min before infection, and the dose was repeated after 5 days.

The experimental results showed (Table 2) that esculamine, like aminoadamantan, inhibits development of influenza pneumonia and alleviates its course. If the dose of virus was 1.5 and 10 LD_{50}, for instance, these substances prolonged the survival period and increased the rate of survival of the mice by 50-100% compared with the control animals. However, if the dose of virus was increased to 50 LD_{50}, the activity of both substances was much lower, as regards both their effect on the length and the rate of survival of the animals. The results of a histological study of the lungs of the control and esculamine-treated animals, carried out by A. M. Prokhorova, confirmed the reduction in severity of the pathological changes (hemodynamic disturbances and inflammatory foci) in the lungs of the treated mice. Esculamine also inhibited reproduction of virus in the lungs, without, however, having a virucidal action.

Comparative study of the effect of esculamine and aminoadamantan on tissue permeability and resistance of the blood vessels showed that if the preparations were administered orally in 5 doses of 60 mg/kg their activity was the same.

It may thus be postulated that esculamine, like aminoadamantan, prevents penetration of virus into the cell and reduces injury to the blood vessel wall in virus infection.

In the third section of the investigation, permeability was studied in certain forms of cardiovascular pathology, and the effect of administration of substances with vitamin P activity in these cases was examined. Observations have been made, mainly in clinical conditions, according to which in certain heart diseases (myocardial infarction, chronic coronary insufficiency, certain disorders of the heart valves) the permeability of the patients' cutaneous vessels is modified [7, 10]. Morphological changes in the myocardium in such conditions also indicate changes of permeability [3, 6]. However, the overall permeability of the myocardium and coronary vessels has not been studied experimentally.

In the authors' experiments coronary insufficiency was produced by creating experimental neuritis of the right vagus nerve in rabbits by injecting resinified terpentine into the nerve [12] and myocardial ischemia was produced by ligating the anterior descending branch of the left coronary artery. The cardiovascular disorders observed after these operations were studied by N. A. Yushchenko electrocardiographically and morphologically [12].

The tissue and vascular permeability were investigated 3 h and 1 and 8 days after injection of terpentine into the right vagus nerve or after ligation of the left coronary artery, i.e., at the time of maximal severity of the disturbances produced. The rabbits were given an intravenous injection of a solution of sulfacyl-sodium (100 mg/kg) or fluorescein (sodium salt, 10 mg/kg), and 20 min later the animal was killed and the concentration of injected indicator (in mg %) was determined in the plasma and myocardium. The coefficient of permeability was calculated. The experimental results are shown in the figure. In experiments with irritation of the vagus nerves the overall permeability of the coronary vessels and myocardium to sulfacylsodium was increased at all three times of investigation.

The results of the experiments to investigate the permeability of the coronary vessels to the fluorescein-protein complex 24 h after operation are shown in the figure. After irritation of the vagus nerve the increase in vascular permeability was not statistically significant. In the case of myocardial ischemia permeability in zones of microscopically unchanged heart muscle was slightly increased, while in the zone

of ischemia the changes of permeability were most marked, a result attributable both to disturbance of permeability of the blood vessels of collateral branches and the vessels of Thebesius in this region, and also to retention of the fluorescein-protein complex by the muscle tissue. A single intravenous injection of esculamine in a dose of 10 mg/kg into rabbits after ligation of the coronary artery reduced permeability to the fluorescein-protein complex only in the region of ischemia. The normal electrocardiographic indices were also restored in these same animals.

Hence, after chronic irritation of the vagus nerve or ligation of the coronary artery the overall permeability of the myocardium and coronary vessels is increased, and it may be reduced by administration of substances with vitamin P activity.

The results described indicate that where the permeability of the microvascular bed is changed in various pathological processes, in the pathogenesis of which these changes may play an essential role, their restoration to normal may be a basic principle of therapeutic measures taken to influence the course of the disease.

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