APPROACHES TO THE MODELING OF PLATELET-INDUCED CEREBRAL MICROEMBOLISM AND THE STUDY OF THE EFFECT OF DRUGS ON IT

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An important role in the pathogenesis of the development of regional ischemia is played by the development of microembolism of the terminal vascular bed by aggregated platelets [2]. Attempts have been made to create a model of regional, including cerebral, ischemia by injecting various agents inducing intravascular platelet aggregation and the development of circulatory disturbances into the blood stream [4, 6]. These investigations have shown that intracarotid injection of inducers of platelet aggregation, such as ADP and arachidonic acid, gives rise to ischemia which involves about 80% of the ipsilateral hemisphere and is clearly demonstrable by electrophysiological investigations, biomicroscopy, and also in the form of disturbances of the acid-base balance (ABB) and energy metabolism of the brain. It has been concluded from the results of research in this direction that this model corresponds most closely to the actual principles of pathogenesis of cerebrovascular disorders. However, systems currently available to record disturbances of the cerebral circulation cannot yet obtain an accurate quantitative evaluation of it, and consequently, cannot be used to assess the effect of drugs on this phenomenon.

This paper describes a model which can monitor the trend of development of disturbances of the cerebral circulation associated with intravascular platelet aggregation and can be used to investigate the effect of drugs on platelet-induced cerebral embolism.

EXPERIMENTAL METHOD

Experiments were carried out on mature cats weighing 2-3.5 kg, anesthetized with urethane and chloralose (600 and 50 mg/kg respectively). The state of the cerebral hemodynamics was studied by recording changes in resistance of the cerebral vessels. Autoperfusion of the cats' brain was carried out through the maxillary artery, as described previously [1]. Parallel recordings were made of the perfusion and arterial pressures (PP and BP respectively) by means of Elema strain-gauge transducers (West Germany). In some experiments synchronous monitoring of the parameters of ABB in the arterial blood was carried out by means of a microanalyzer (Radiometer, Denmark). Soluble collagen (Dade, USA), which is superior to the ADP and arachidonic acid used in other investigations because of the absence of the vascular component in the ultimate effect on the cerebral hemodynamics, was used as inducer of intravascular aggregation. Collagen was injected in a volume of 0.5-1.0 ml (20-200 μg/kg body weight) directly into the perfusion line; the duration of the injection was 0.5-1 min. The drugs to be tested were injected in the same way. Prostacycline (PGI_2) was used in the form of an intravenous infusion (2-5 min) in a concentration of 50-500 $ng \cdot kg^{-1} \cdot min^{-1}$, and was injected by means of an automated, programed Perfusor E injector (West Germany). Preparations for long-term PGI_2 infusion were made in accordance with the method in [7]. The data were subjected to statistical analysis by Wilcoxon's paired test and the Wilcoxon–Mann–Whitney nonparametric test.

EXPERIMENTAL RESULTS

The terminal vascular network of the brain resembles a giant filter, through which blood passes through the action of the propulsive forces of a perfusion pump, working under constant volume conditions. It is clear that if some microvessels of the brain are blocked by

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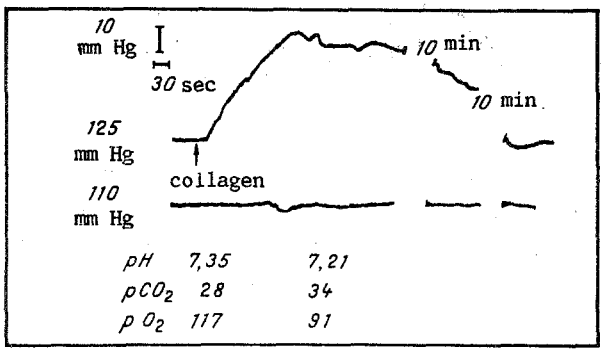

Fig. 1. Effect of intracarotid injection of collagen (100 μg/kg) on PP and BP of a cat and on parameters of ABB of the arterial blood.

platelet aggregates, this will quickly be manifested as an increase in PP, and the greater the proportion of occluded vessels, the greater this increase will be. According to this principle, which is in agreement with the classical filtration method of investigation of aggregation [2], the model described below was created.

Injection of collagen caused a rapid rise of PP, which stabilized within quite a short time at a high level without any significant changes of BP (Fig. 1). The degree and duration of the changes in PP depended on the dose of collagen: with a dose of 50 ng·kg^{-1}·min^{-1} the increase in PP was 27.3/17.3 — 37.3 mm Hg, and the time for it to stabilize at a high level was about 5-20 min; with a dose of 100 ng·kg^{-1}·min^{-1}, however, the corresponding figures were 40.4/22.6 — 58.2 mm Hg and 15-40 min. It must, however, be pointed out that no absolute dependence of the degree of development of platelet-induced microembolism on the dose of collagen could be observed. The reason is probably that the effect also depended on the initial state of the cerebral microcirculation and, in particular, on the ability of the platelets to aggregate, sensitivity to collagen, and so on. It will also be noted that after injection of collagen, especially in the initial period, more or less conspicuous fluctuations of PP, evidently connected with movement of the platelet aggregates in the cerebral capillaries and, in particular, with the release of vasoactive factors, mainly vasoconstrictors, from the platelets, giving rise to various vasomotor responses, could be observed. All these processes are accompanied by metabolic disturbances, manifested as changes in pH of the arterial blood toward acidosis and changes in its gas composition (Fig. 1). It can be tentatively suggested that these changes were much more marked actually in the brain tissue.

Consequently, changes in PP can be used as a quantitative measure of the level of platelet-induced cerebral microembolism. Their transient character, as shown by biomicroscopy of the pial vessels [6], is associated with spontaneous disaggregation of the platelet aggregates in the microvessels and their "propulsion" through them. The duration of development of the circulatory disturbances could be controlled to some degree by the dose of collagen and also by the time of its administration. In the case of intracarotid injection of collagen, the brain acts as a filter, not allowing platelet aggregates to pass into the systemic circulation, so that microembolism of other organs cannot develop. If collagen was injected intravenously (into the femoral vein) a rise of PP was observed, but it was 40-50% less than that observed with intracarotid injection. Meanwhile marked respiratory disturbances, fluctuations of BP, and disturbances of cardiac activity developed. In one case the animal died from acute cardiopulmonary failure, as a result of systemic microembolism of the heart and lungs.

The effect of some vasotropic drugs on the cerebral circulation was analyzed on the above model during the development of cerebral ischemia induced by intravascular platelet aggregation. The action on PP is shown schematically in Fig. 2. Papaverine and cavinton were found to produce a transient fall in the resistance of the cerebral vessels, but under these circumstances PP soon returned to its high level again, evidence of absence of recanalization of the vascular bed of the brain under the influence of these drugs. Had recanalization taken place under their influence, PP would have returned to a new level more or less close to that before injection of collagen. To characterize the "recanalizing" effect of the drugs on the cerebral circulation under these conditions, it is therefore possible to use a parameter reflecting the degree of recovery of the PP level, when raised by collagen, after being temporarily lowered under the influence of this drug (Table 1). It will be clear from Table 1 that

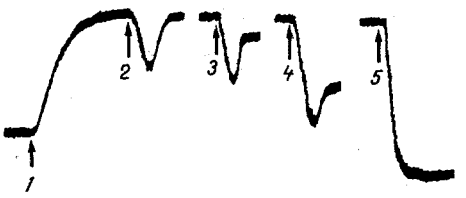

Fig. 2. Scheme showing effect of papaverine (2), cavinton (3), verapamil (4), and PGI_2 (5) on PP, artificially raised by injection of collagen (1).

TABLE 1. Effect of Drugs on PP under Conditions of Platelet-Induced Microembolism

Drug	Degree of lowering of PP, mm Hg	Degree of restoration of high level of PP, %
Papaverine (1 mg/kg)	14,2 (6,9—21,5)	106,3 (90,6—122,0)
Cavinton (0.5 mg/kg)	20,8 (12,4—29,2)	88,5 (71,9—105,1)
Verapamil (0.25 mg/kg)	19,4 (9,9—28,9)	56,9 (26,1—87,7)
PGI_2 (250 ng·kg^{-1}·min^{-1})	35,3 (25,4—45,2)	4,8 (1,5—8,2)
PGI_2 (50 ng·kg^{-1}·min^{-1} + verapamil (0.25 mg/kg)	34,8 (19,1—50,5)	7,9 (2,8—13,0)

under the influence of papaverine recovery of the capillary perfusion of the brain virtually did not take place, whereas under the influence of cavinton it recovered very slightly. When the calcium antagonist, verapamil, was used, recovery of the high PP level was partial in character, but infusion of PGI_2 (250 ng·kg^{-1}·min^{-1}) led after only 2 min to a distinct fall of PP, which eventually flattened out at the level existing before injection of collagen (Fig. 2, Table 1). This kind of effect of PGI_2 is evidently linked with the fact that, although it is not weaker than the other drugs in its vasodilator action, it is much stronger than they are in its ability to induce platelet disaggregation, and to block the sensitivity of the platelets to aggregation inducers [2]. Under these circumstances infusion of PGI_2 in a lower concentration (50 ng·kg^{-1}·min^{-1}) caused no significant changes in PP or recanalization of the cerebral microvessels when blocked by aggregates, but after its infusion for 3 min in this dose the character of the effect on these values of the parameters of verapamil changed. As Table 1 shows, under these conditions the ability of verapamil to lower PP was increased and the degree of its restoration to the previous high level was reduced. This agrees with previous data indicating positive interaction of calcium antagonists and PGI_2 in the realization of their vascular and platelet-induced effects [3].

Another approach to the study of the effect of drugs on platelet-induced embolism may be to evaluate their ability to prevent the rise of PP under the influence of collagen. For instance, against the background of infusion of PGI_2 for 2 min, injection of collagen caused no significant changes in cerebrovascular resistance; moreover, this effect of PGI_2 was exhibited in a dose as low as 50 ng·kg^{-1}·min^{-1}.

The results demonstrate that the cerebrovascular effects of drugs with a purely vascular mechanism of action are unsatisfactory when there is an imbalance between the vascular network of the brain and the blood. The unsatisfactory results of treatment of cerebrovascular disorders with vasodilator drugs can evidently be attributed in some degree to this fact. Meanwhile compounds acting both on the blood vessels and platelets, such as verapamil and PGI_2, are sufficiently effective under these conditions also and lead not simply to transient vasomotor responses, but also to restoration of circulatory homeostasis of the brain in full measure.

The suggested approach thus enables new elements in the mechanisms of action of drugs to be evaluated: their ability to normalize the cerebral circulation under conditions of platelet-induced microembolism.

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MODELS OF OVARIAN TUMORS IN RATS

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Tumors of the ovary occupy a leading place among neoplasms in women. Because of the difficulty of early diagnosis, malignant ovarian tumors are found only in the late stage of development, and the results of treatment are still unsatisfactory. The solution of this problem may perhaps be linked to a certain extent with the elucidation of the pathogenesis of this neoplasm. The pathogenesis of ovarian tumors is a complex process which is under the influence of various neuroendocrine disturbances. The study of some of these aspects is possible only on experimental models. Various transplantable strains, corresponding to clinical cases only in their morphology, are widely used as models of malignant neoplasms in experimental oncology. Investigations on human tumors, transplanted into athymic animals, do not always help to shed light on whether disturbances of the endocrine system are the cause or effect of the development of ovarian neoplasms. The most suitable experimental model with which to study the role of neuroendocrine changes in the development of ovarian neoplasms is induced tumors. Various methods of obtaining ovarian tumors in rats have been described in the literature [3, 7].

The aim of this investigation was to compare experimental ovarian neoplasms induced in rats by different procedures.

EXPERIMENTAL METHOD

Experiments were carried out on 486 noninbred female rats reared at the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. The distribution of the animals by groups is shown in Table 1. In group 1, a fragment of ovary was autografted beneath the capsule of the spleen in castrated rats aged 1.5 months. Rats of group 2 underwent subtotal castration at the age of 2 months, and rats of group 3 were irradiated with x-rays (200 R) in the lumbar region. The rats of group 4, aged 16-20 days, received a single intraperitoneal injection of nitrosoethylurea (NEU) in a dose of 30 mg/kg, whereas the rats of group 5 received NEU in a dose of 10 mg/kg by the transplacental route on the 21st day of embryogenesis. The animals of group 6, at the age of 2 months received a subcutaneous injection of 1,2-dimethylhydrazine (DMH) in a dose of 2 mg/kg. Rats of group 7 received testosterone propionate in a dose of 1 mg on the last 3 days of embryogenesis. Experimental groups 1, 6, and 7 had their own controls; common controls were provided for groups 2 and 3 and for groups 4 and 5. The animals were regularly inspected and rats in a poor condition were killed with ether. All the animals were autopsied, and the ovaries of the experimental and control

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