EFFECT OF ACETYLSALICYLIC ACID AND PENTOXIFYLLINE (TRENTAL) ON INTRAVASCULAR ERYTHROCYTE AGGREGATION STIMULATED BY ARACHIDONIC ACID

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UDC 615.212.3.015.45:612.111.44

KEY WORDS: erythrocyte aggregation; arachidonic acid; acetylsalicylic acid; pentoxifylline.

Much evidence of the extremely important role of arachidonic acid in platelet aggregation has recently been published [10]. The aggregating action of agents such as thrombin and collagen, in the opinion of some workers, is realized through liberation of arachidonic acid from phospholipids of platelet membranes [11], followed by the formation of intermediate products of synthesis of prostaglandins E_2 and $F_{2\alpha}$, namely endoperoxides H_2 and G and thromboxane A_2 [6]. Many investigators ascribe the decisive role in platelet aggregation to these labile compounds [6]. It has also been shown that arachidonic acid itself induces platelet aggregation in experiments *in vitro* and death of the experimental animals from thrombosis after intravenous injections [12, 13].

However, whereas the role of arachidonic acid in platelet aggregation is currently being intensively studied, its possible participation in erythrocyte aggregation has been almost totally neglected. Yet this is an important problem because the increased content of arachidonic acid in the composition of nonesterified fatty acids in various stress situations could alter the rheologic properties of the blood. On the basis of results obtained in the present writers' previous experiments, it was postulated that platelet and erythrocyte aggregation shares the same mechanism [1-3]. Experiments *in vitro* showed that arachidonic acid causes aggregation not only of platelets, but also of erythrocytes. Under these circumstances aggregation of the erythrocytes is as a rule accompanied by their hemolysis [1].

In the investigation described below the role of arachidonic acid in intravascular erythrocyte aggregation and the effect of acetylsalicylic acid (aspirin) and pentoxifylline on this process was studied in experiments *in vivo*.

EXPERIMENTAL METHOD

Experiments were carried out on 25 rats weighing 180-200 g anesthetized with pentobarbital. Aggregation of erythrocytes in the mesenteric vessels was produced and the process recorded photographically by means of an apparatus for intravital microscopy mounted on the MBI-6 microscope. Arachidonic acid (from Merck-Schuchardt, West Germany) was dissolved in 96° ethanol. The whole volume of arachidonic acid (0.2 ml), in a mean dose of 3-5 mg/kg body weight, was injected into the caudal vein in the course of 2 sec. According to data in the literature, the concentration of arachidonic acid used causes death of experimental animals [13]. The animals were divided into four groups: 1) 10 rats receiving arachidonic acid only; 2) five rats receiving aspirin *per os* in a dose of 100 mg/kg in the form of a 1% suspension in starch gel before injection of the arachidonic acid; 3) five rats which received an intravenous injection of pentoxifylline in a dose of 20 mg/kg before the injection of arachidonic acid; the five rats of group 4 (control) received an intravenous injection of 96° ethanol (0.2 ml). The state of the microcirculation was studied in the animals of groups 1, 2, and 3 in the course of 30 min before the injection of arachidonic acid.

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0007-4888/80/9009-1187$07.50

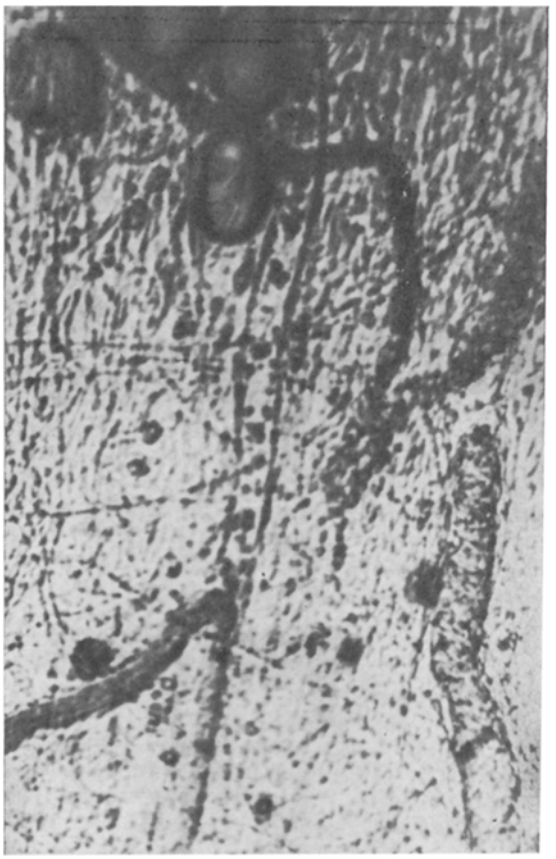

Fig. 1

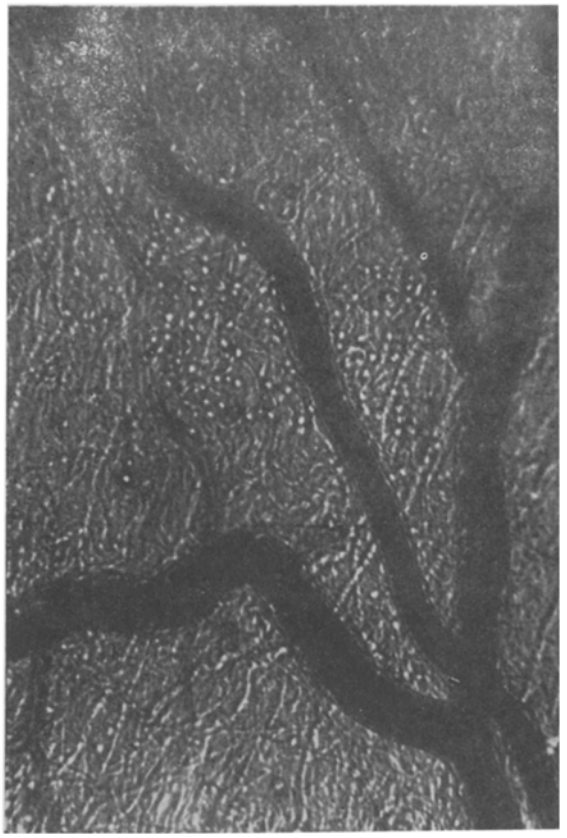

Fig. 2

Fig. 1. Photomicrograph of mesentery of small intestine after injection of arachidonic acid into a rat. Here and in Fig. 2, 560 ×.

Fig. 2. Photomicrograph of mesentery of small intestine of a rat after injection of arachidonic acid 15-20 min after preliminary injection of pentoxifylline.

In the experiments *in vitro* the effect of aspirin on erythrocyte aggregation stimulated by arachidonic acids was studied *in vitro*. Aggregation was studied by a photometric method [5] in an aggregometer, using a suspension of erythrocytes (1:400) in physiological saline. The degree of aggregation was assessed from the maximal amplitude of aggregation (M_{max}). The final concentration of arachidonic acid in the experiments *in vitro* was 45 mM. The aspirin solution was prepared by the method of Ulutin et al. [15].

EXPERIMENTAL RESULTS

In all animals of groups 1, 2, and 3 no appreciable changes in the microcirculation were observed before injection of arachidonic acid. In the animals of group 1 considerable slowing of the blood flow, the appearance of large aggregates of erythrocytes, and zones of stasis was observed immediately after injection of arachidonic acid (Fig. 1). In some segments of the microvessels intravascular hemolysis was observed and the vessels became pink in these segments. Death of most animals took place 2-3 min after injections of these doses of arachidonic acid. Immediately after death of the animals, hemolysis of a considerable proportion of the erythrocytes was observed visually. In animals which did not die after injection of arachidonic acid, the same picture as initially was observed: rapid slowing of the blood flow, aggregation of erythrocytes, and hemolysis. However, the velocity of the blood flow gradually was restored and reached its initial level after 15-30 min.

In the animals of group 2 which received aspirin 2 h before injection of arachidonic acid, no aggregation or hemolysis of erythrocytes took place. However, despite the absence of aggregation, these animals died much sooner than those in the previous series of experi-

TABLE 1. Effect of Aspirin on Erythrocyte Aggregation Stimulated by Arachidonic Acid (M ± m)

Agent	Experiment No.	M_{max}, mm	P
Arachidonic acid	11	88,0±4,03	
Arachidonic acid + aspirin	9	49,2±5,3	<0,001

ments, namely in the course of 10-15 sec, whereas hemolysis of the erythrocytes in the vessels was not observed until 2-2.5 h after death.

In the animals of group 3, which received an injection of pentoxifylline 2 h before the experiment, just as in the rats of group 2, no aggregation or hemolysis of erythrocytes was observed. However, the animals of this group also died in the course of 10-15 sec. Injection of pentoxifylline 15-20 min before the experiments did not prevent aggregation and hemolysis of the erythrocytes under the influence of arachidonic acid, but it likewise was not followed by immediate death of the animals (Fig. 2).

In the animals of the control group, receiving ethanol only, no visible changes could be seen in the microcirculation.

These results are evidence that arachidonic acid causes intravascular erythrocyte aggregation, hemolysis, and death of the animals. Preliminary injections of acetylsalicylic acid or pentoxifylline abolish this effect. These substances evidently stabilize the erythrocyte membranes and so prevent lysis of the cells.

Experiments *in vitro* also showed that aspirin largely prevents erythrocyte aggregation stimulated by arachidonic acid and also retards and reduces the development of hemolysis (Table 1).

Most investigators consider that the aggregating action of arachidonic acid is associated with the formation of prostaglandin G_2 and H_2 endoperoxides and of thromboxane A_2 [6]. However, more recently data have been obtained in various laboratories [8, 9] independently, including in our own [2], to show that intermediate products of prostaglandin synthesis do not play the decisive role in the aggregation even of blood cells such as platelets. They may play an even less important role in the aggregation of other blood cells such as erythrocytes, which are known not to possess enzyme systems synthesizing prostaglandins [7, 2]. The leading role in the mechanism of action of arachidonic acid on the aggregation of platelets and erythrocytes may evidently be played by lipid–protein transformation of the blood cell membranes on account of excessive incorporation of arachidonic acid into the phospholipids of these membranes [4].

Aspirin, which is fixed in large quantities on the lipid and protein fractions of membranes, can evidently prevent this process and thereby prevent aggregation and subsequent hemolysis of erythrocytes under the influence of arachidonic acid. The action of pentoxifylline may be linked with its inhibitory effect on phosphodiesterase and the consequent accumulation of cyclic AMP. This action of pentoxifylline has recently been studied on platelets [14]. According to data in the literature, cyclic AMP itself prevents the aggregating effects of arachidonic acid on platelets, although prostaglandin synthesis is preserved under these conditions [9].

The results of experiments *in vivo* thus confirm our results obtained *in vitro*, showing the very important role of arachidonic acid in aggregation and lysis of erythrocytes.

The fact that arachidonic acid, injected after preliminary administration of aspirin and pentoxifylline, still causes rapid death of the animals despite absence of aggregation and lysis of the erythrocytes, which the present investigation has shown, deserves special attention. It can only be suggested that under these conditions (when the circulation is undisturbed) the sudden arrival of arachidonic acid in high concentrations at the heart may lead to instant cardiac arrest as a result of blocking of the contractile function of the heart.

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ASSESSMENT OF THE STATE OF THE LUNG SURFACTANT SYSTEM IN HYPOXIA WITH PARAPULMONARY BLOOD OXYGENATION

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UDC 616-008.922.1-008.64-07:612.212.014.1.462.8

KEY WORDS: hypoxia; dogs' lungs; membrane oxygenator.

In the modern view, surface-active components of the phospholipid complex constituting the basis of the lung surfactant system (LSS) are responsible for the stability of the alveoli of the lungs. The study of LSS is important when the extrapulmonary gas exchange is maintained by means of membrane oxygenators (MO). There is reason to suppose that the "safety" of artificial support systems for the body can be judged to a certain extent from the degree of change in the LSS [3, 4].

The object of this investigation was to study the state of the LSS in animals with severe hypoxia of respiratory type, incompatible with life, and after arteriovenous connection to a Soviet "Sever-OMR" oxygenator for the purpose of treatment.

EXPERIMENTAL METHOD

Experiments were carried out on 18 mongrel dogs weighing 13-25 kg under morphine-hexobarbital anesthesia with muscle relaxation and artificial ventilation. The dogs were divided into three groups (with six animals in each group). The dogs of group 1 were provided with adequate artificial ventilation of the lungs. The animals of group 2 were on hypoventilation (respiration rate 3-4 breaths/min, respiratory minute volume 40% of normal); an arteriovenous shunt was created between the femoral artery and vein, with a volume velocity of blood flow of not more than 1 liter/min. The dogs of group 3 also were artificially hypoventilated. A type Sever-OMR MO was incorporated into the arteriovenous shunt [1]. The gas-exchange system functioned for 4 h.

The lung surfactant was investigated in broncho-alveolar washings and pieces of lung tissue. The washings were obtained by means of a modified double-barreled catheter of Fogarty

P. A. Kupriyanov Postgraduate Surgical Clinic, S. M. Kirov Military Medical Academy, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Kolesov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 9, pp. 279-281, September, 1980. Original article submitted September 17, 1979.

 0007-4888/80/9009-1190$07.50