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PLATELET ACTIVATING FACTOR AND ENDOTOXIN-INDUCED PLATELET ACTIVATION

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UDC 612.111.7.014.46].063.08

KEY WORDS: endotoxin-induced platelet activation; platelet aggregation

The possibility that platelet activating factor (PAF) participates in endotoxin-induced platelet activation has virtually not been studied. Yet its generation and release from activated leukocytes and zones of inflammation suggest that the presence of PAF in the blood may play a definite role in platelet activation in the thrombohemmorrhagic syndrome, which accompanies infectious diseases. Meanwhile the endotoxinemia in infectious pathology creates special conditions for activation of the blood cells, for it has been shown that endotoxins are inducers of aggregation and secretion of platelets [1, 2, 3]. The high sensitivity of platelets to PAF [7] and to endotoxins [4] may contribute to the fact that during their interaction subthreshold concentrations, which themselves do not give rise to any significant activation of cells, become sufficient to stimulate cell functions. Interaction of this kind between PAF and arachidonic acid and between PAF and collagen has been demonstrated experimentally [6, 7].

To elucidate the role of PAF in endotoxin-induced platelet aggregation, experiments were carried out in vitro in which endotoxin-induced platelet aggregation was recorded before and after incubation of platelets with PAF and also during exposure to the combined action of PAF and endotoxin.

EXPERIMENTAL METHOD

Experiments were carried out on platelet-enriched plasma (PEP) obtained from blood donors. Blood stabilized with 3.8% sodium citrate solution was centrifuged for 10 min at 1500 rpm. The number of platelets in 1 μl of plasma was counted and their number adjusted to 250,000-300,000 /μl with platelet-deprived plasma. The aggregating capacity of the platelets [5] was recorded by means of an aggregometer (made by the experimental workshops of the Academy of Medical Sciences of the USSR). ADP (10^{-3} %), PAF (in concentrations of 10^{-14} to 10^{-6} M), menigococcal B lipopolysaccharide (LPS) (from 0.1 to 1 μg/ml), and *Salmonella typhimurium* LPS (10-20 μg/ml) were used to induce aggregation. The meningococcal LPS was obtained in the Laboratory of the Central Research Institute of Epidemiology, Ministry of Health of the USSR, the *S. typhimurium* LPS from "Sigma" (USA), and the PAF from "Serva" (West Germany). Addition of the aggregation inducers to PEP in different orders was carried out with continuous mixing and recording of the aggregation process, so that the effect of each of them and also the possibility of their interaction, on platelet activity could be studied. In control experiments, instead of aggregation inducers, corresponding volume of physiological saline was added. The degree of irreversible ADP-induced platelet aggregation served as the control value (100% aggregation). On the addition of one of the inducers, the aggregation process was recorded until the curve flattened out on a plateau, and only when this had occurred was the next added.

EXPERIMENTAL RESULTS

According to data obtained by several workers [6-8], PAF potentiates the action of other inducers (ADP, thrombin, collagen) in weak concentrations, but in high concentrations it does not affect the response of the cells to these inducers.

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. I. Pokrovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 5, pp. 538-540, May, 1989. Original article submitted March 18, 1988.

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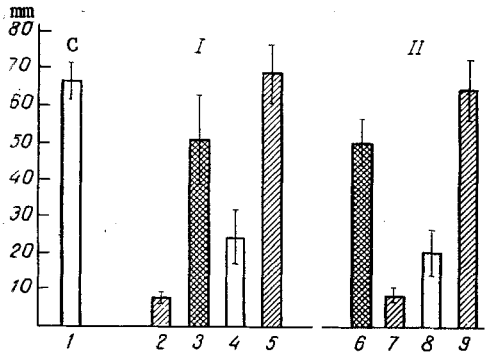

Fig. 1. Degree of platelet aggregation induced: 1) by ADP (10^{-3} %) – control. Variant a: 2) by meningococcal B LPS (1.0 μg/ml); 3) by PAF (10^{-6} M), 4) by ADP (10^{-3} %); 5) combined effect of LPS + PAF + ADP; variant b: 6) by PAF (10^{-6} M), 7) by LPS (1.0 μg/ml), 8) by ADP (10^{-3} %), 9) combined effect of PAF + LPS + ADP. Ordinate, degree of platelet aggregation (in mm).

To study interaction between PAF and endotoxin, the appropriate concentration of PAF had to be chosen. For this purpose the degree of platelet aggregation induced by different concentrations of PAF was determined.

According to our data, during the action of PAF in concentrations of between 10^{-14} and 10^{-9} M the degree of platelet aggregation remained at the same level. With PAF in a concentration of 10^{-8} M the degree of platelet aggregation increased somewhat, to 17% of normal on average. A further increase in the PAF concentration to 10^{-6} M led to a sharp increase in the degree of aggregation (about 90% of the normal level).

To study interaction between PAF and endotoxins in the process of platelet aggregation the following concentrations of PAF were used: 10^{-10}-10^{-9} M and 10^{-7}-10^{-6} M.

In 17 experiments, to determine the action of high concentrations of PAF on endotoxin-induced platelet aggregation, meningococcal LPS (1.0 μg/ml) was added first to the samples of PEP, PAF was added next, and ADP last. Three waves of aggregation were recorded: they amounted to 8.5 ± 0.9 mm, 49.4 ± 10.3 mm, and 23.0 ± 7.2 mm respectively. The combined effect of the three inducers was 72.3 ± 7.6 mm (Fig. 1, I).

Changes in the order of addition of the inducers to the samples of PEP did not change the magnitude of response of the cells to each of them, or the combined effect: the degree of platelet aggregation induced by PAF was 46.9 ± 5.1 mm, by LPS 8.1 ± 2.1 mm, and by ADP 20.0 ± 6.5 mm; the combined effect of the three inducers was 67.7 ± 6.2 mm (Fig. 1, II).

In the next series of the experiments, PAF and endotoxins were added simultaneously to PEP in the concentrations indicated above. The combined effect of PAF and the endotoxins did not differ significantly from the combined effect when they were added consecutively or when they were separately ($p > 0.05$). Independence of the level of response of the cells of the order of addition of the aggregation inducers to the samples of PEP with high doses of PAF also was observed when its effect was studied on aggregation induced by *S. typhimurium* LPS.

In 15 experiments to study the action of low concentrations of PAF on endotoxin-induced platelet aggregation, PAF was added to PEP in a concentration of 10^{-10}-10^{-9} M. This dose of activator induced platelet aggregation by an average degree of 10.6 ± 0.7 mm (Fig. 2, I).

In the next series of experiments (18) the degree of aggregation of platelets after addition of *S. typhimurium* LPS to PEP averaged 10.8 ± 0.9 mm (Fig. 2a, II).

On successive addition, first of PAF and then of endotoxin to the PEP the response of the cells to the latter was the same as when no PAF was added beforehand (11.0 ± 0.9 mm; Fig. 2, III).

Simultaneous addition of PAF and LPS to PEP led to a more marked degree of platelet

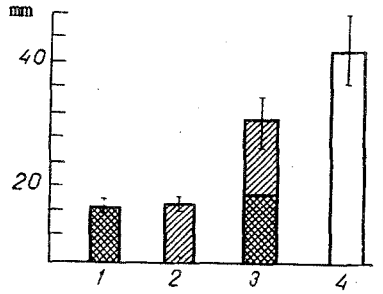

Fig. 2. Degree of platelet aggregation induced by: variant a: I) PAF (10^{-9} M), II) *S. typhimurium* LPS (20 μg/ml), III) LPS after PAF, IV) PAF + LPS simultaneously; variant b: I) PAF (10^{-10} M, II) meningococcal B LPS (1 μg/ml), III) LPS after PAF, IV) PAF + LPS simultaneously. Ordinate, degree of platelet aggregation (in mm). Dots indicate separate experiments.

aggregation (in 8 of 14 experiments, see Fig. 2, IV). The combined action of PAF and endotoxin induced aggregation, the degree of which exceeded the combined effect when each inducer was added separately (by 23%), and also the combined effect when they were added consecutively (by 36%).

Similar results also were obtained by the combined action of low concentrations of PAF and meningococcal B LPS. In this case the maximal effect was observed in cases when below-threshold concentrations were used.

These results are evidence that the simultaneous action of low concentrations of PAF and endotoxins of Gram-negative bacteria (*S. typhimurium* and *Meningococcus*) has a stronger effect on the aggregating capacity of human platelets than that of each of the inducers separately.

We know [6, 7] that below-threshold concentrations of PAF, arachidonic acid, and collagen, inducing very weak platelet aggregation, can give rise to quite pronounced aggregation by the combined action of PAF and arachidonic acid or of PAF and collagen.

In low concentrations PAF is considered [8] to activate platelets by a thromboxane-A_2-dependent mechanism, whereas in high concentrations, the activation is independent.

In our experiments the combined action of PAF in low concentrations and of endotoxins was evidently more effective also, for the arachidonic acid cascade also was involved in the reaction, leading to the formation of TXA_2. The possibility of such a mechanism is based on an earlier study [3] which showed that preincubation of platelets with arachidonic acid enhances their response during LPS-induced aggregation.

Thus each of the inducers studied has its specific receptors on the platelet plasma membrane, for the number of platelets involved in the response is about equal regardless of the order in which the inducers are added to PEP. Meanwhile, in the high concentrations which we tested, PAF is not a mediator of endotoxin-induced platelet aggregation.

In low concentrations the combined action of PAF and endotoxins on platelets is more effective than that of each inducer separately, or when added consecutively.

Considering the special conditions created by endotoxinemia, when endotoxins and PAF appear simultaneously in the blood, their interaction in low concentrations can facilitate platelet activation and aggregation, thus making a disturbance of the microcirculation more likely even in the initial period of the disease.

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EFFECT OF NALOXONE ON CIRCULATORY CHANGES IN ANAPHYLACTIC SHOCK

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UDC 616.001.36-056.3-07:616.16-008.1-02:615.214.31

KEY WORDS: anaphylactic shock; circulation of the blood; naloxone

Administration of naloxone improves the state of animals in shock whether induced by pain, hemmorrhage, exotoxins, and endotoxins or of spinal origin [8-10], although the effect achieved depends on the dose of the drug and the stage of the pathological response [1, 7]. Since one cause of shock is blockage of the microcirculatory bed [2] and since it is essential to study the specific features of its different types [4], and since the action of antagonists of opiate peptides in analphylactic shock (AS) has not yet been analyzed, it was decided to study circulatory changes in AS in relation to the effect of naloxone.

EXPERIMENTAL METHOD

Guinea pigs weighing 250 g were sensitized by two subcutaneous injections of horse serum in a dose of 0.1 mg/kg. The reacting dose (0.5 mg/kg) of serum was injected 3 weeks later into the heart. A group of animals was given a subcutaneous injection of naloxone in a dose of 0.5 mg/kg 0.5 h before injection of the allergen. Injection of allogeneic serum led to the development of AS at once or after 1-2 min in all the guinea pigs. Many of them died in the first 10 min of the experiment, others later; the remainder which survived were killed at various times (from 1 h to 30 days) after development of AS. Data on mortality of the animals from AS, depending on the action of naloxone, were subjected to correlation analysis with calculation of the coefficient of cross-correlation [5]. The lungs, liver, kidneys, heart, adrenals, spleen, stomach, and pancreas of the experimental, and also of control (not sensitized, killed after injection of allogeneic serum) guinea pigs were studied histologically in sections stained with hematoxylin-eosin, azure-eosin, and toluidine blue. The microcirculatory bed also was visualized in film preparations of the mesentery by

TABLE 1. Survival Rate of Guinea Pigs from AS Depending on Action of Naloxone

Experimental conditions	Number of animals	
	which died	which survived longer than 1 h
AS induced without naloxone	40	6
AS induced 30 min after naloxone	15	16

Department of Pathological Anatomy, Yaroslavl' Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 5, pp. 540-542, May, 1989. Original article submitted March 25, 1988.

 0007-4888/89/0005-0614$12.50