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MORPHOLOGICAL ANALYSIS OF CHANGES INDUCED IN THE LUNGS AND KIDNEYS BY ACETYLGLYCERYL PHOSPHORYLCHOLINE ESTER AND LIMITED BY VERAPAMIL

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The acetylglyceryl ester of phosphorylcholine (1-alkyl-2-acetyl-sn-glycero-3-phosphocholine), known in the literature as platelet activation factor (PAF), plays an important role in inflammation, shock, and disturbances of hemostasis. Although discovered initially as a mediator of IgE-dependent anaphylaxis, it is probably a universal signal for activation of cells and an endogenous mediator of damage in various pathological processes, proceeding on an immune basis [1, 2]. Investigations in vitro have shown that the mechanism of cellular activation by PAF is connected with the rapid opening of calcium channels and a change in the intracellular calcium homeostasis, followed by the release of secondary mediators [4]. PAF has a varied systemic and local action, for it can induce hypotension, and can increase vascular permeability and edema, intravascular aggregation of platelets and neutrophils, chemotaxis, and tissue infiltration by monocytes [8].

In experiments with a single intravenous injection of purified PAF, characteristic shock reactions developed after doses of between 100 and 2000 ng/kg, and their intensity depends on the dose, species, and age of the experimental animals [5, 6]. Morphological and functional changes in the myocardium, lungs, and kidneys in response to small and average doses of PAF are reversible. The blood level of secondary mediators of inflammation and of clotting factors returns to normal under these circumstances; platelets and neutrophils, aggregated and sequestered in the microcirculatory bed, become taken up in the systemic blood flow. Large doses of this mediator lead to death of the animals from severe circulatory disorders [5]. Because of these properties of PAF, changes within target organs in response to long-term exposure to PAF in vivo still remain unexplained. The involvement of calcium channels in the realization of this action likewise has not been adequately studied.

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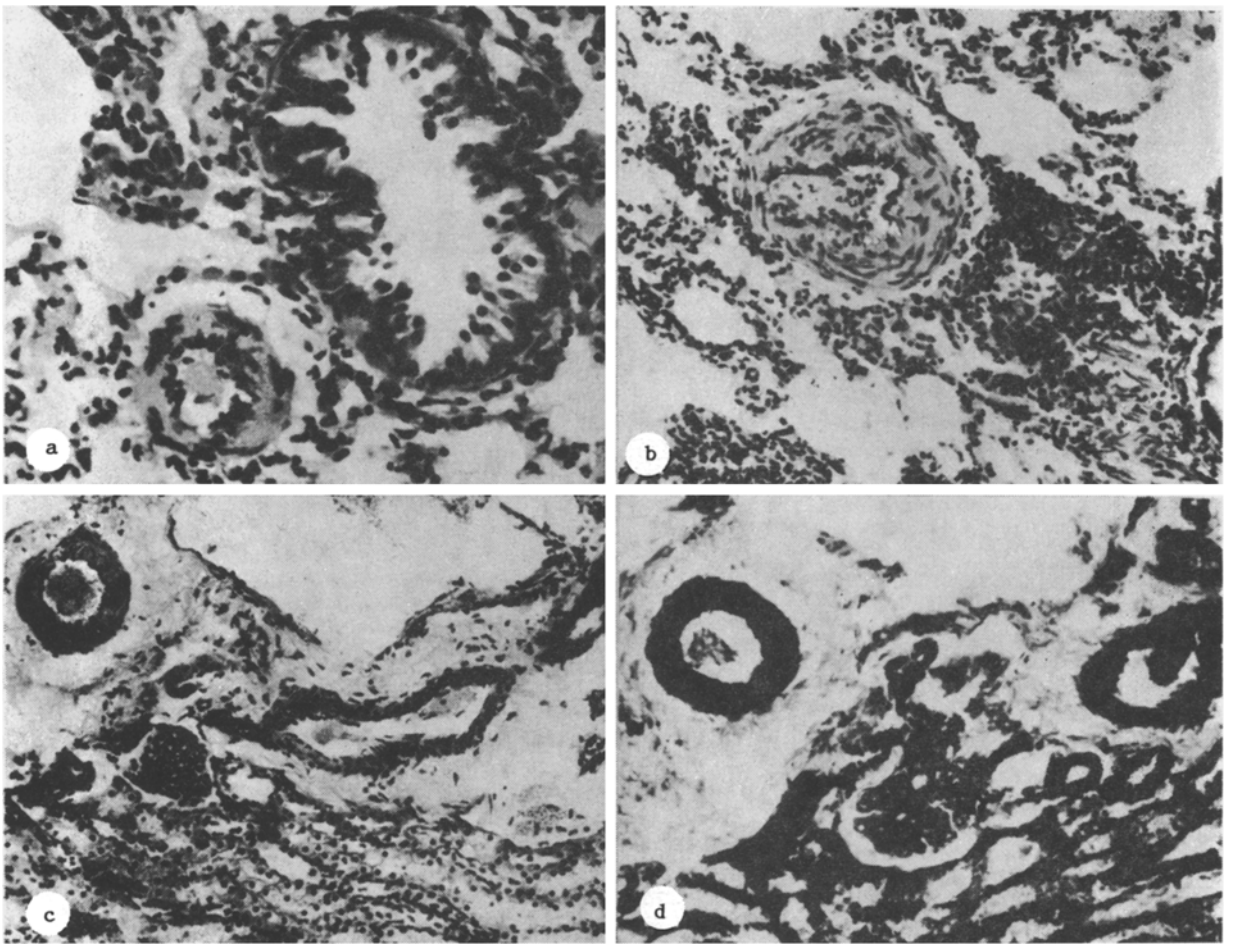

Fig. 1. Structural changes in lungs (a, b) and kidneys (c, d) under the influence of PAF. a) Thrombus in lumen of arteriole; moderate peribronchial infiltration, signs of bronchospasm. Stained with hematoxylin and eosin. 250×; b) pulmonary arteriole in spasm, with lumen obliterated by a red thrombus, intensive perivascular infiltration. Hematoxylin and eosin, 160×; c) proliferation of mesangial cells in glomerulus, edema of stroma, arteriole in spasm containing red thrombus. Hematoxylin and eosin. 160×; d) moderate spasm of renal vessels with juxtamural thrombus. Strongly positive reaction for fibrin in glomeruli and wall of vessels in cellular tissue. Stained for fibrin. 160×.

The aim of this investigation was to undertake a morphological analysis of changes arising in the lungs and kidneys after repeated intravenous injections of synthetic PAF and to study under these conditions the effect of preliminary injection of the calcium channel blocker, verapamil.

EXPERIMENTAL METHOD

Experiments were carried out on male rabbits aged up to one year. The five animals of group 1 received an injection of PAF alone, the five animals of group 2 received PAF with verapamil, and the four animals of group 3 served as the control. Synthetic PAF, namely 1-hexadecyl-2-acetyl-sn-glycerol-3-phosphocholine was obtained from "Boehringer Mannheim" (Austria); 0.5 mg of the compound was dissolved in 1.0 ml of 96.6° ethyl alcohol. Aliquots for injection were then diluted with sterile physiological saline in a volume of 1.0 ml per injection; the final ethanol concentration did not exceed 0.1%. Verapamil solution ("Orion," Finland) also was used, and was diluted when necessary with sterile physiological saline wp to a volume of 1.0 ml per injection. The preparations were injected into the marginal vein of the animals' car in the course of 30 sec 4 times a day with intervals of 3 h between injections; verapamil was given in a dose of 0.5 mg/kg 3 min before the injection of PAF. On the first day the dose of PAF was 100 ng/kg, on the second day 250 ng/kg, on the third day 500 ng/kg, and on the fourth

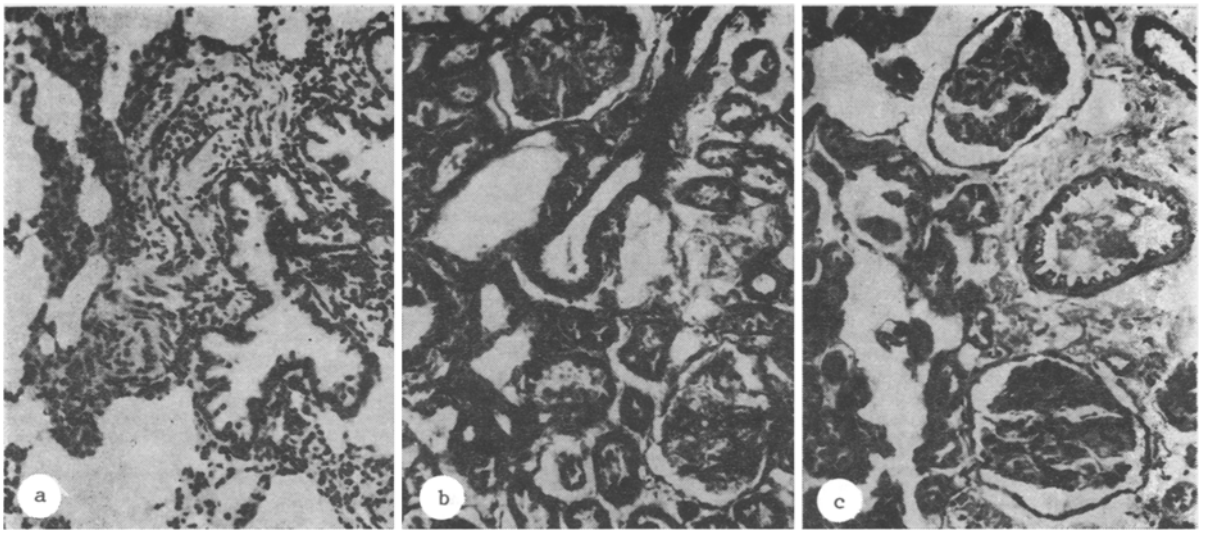

Fig. 2. Effect of verapamil on structural changes in lungs (a) and kidneys (b, c), caused by PAF. a) Dilated and twisted bronchus; congested vessel, signs of perivascular infiltration. Hematoxylin and eosin. 160×; b) distinct outlines of vessel walls in interstices of kidney, absence of fibrin deposits in capillaries of globerumli. Stained for fibrin. 160×; c) dilated lumen of vessel, pycnosis of endothelium, signs of edema in interstices of kidney. Hematoxylin and eosin. 160×.

day 750 ng/kg. The plasma fibrinogen level of the animals also was determined. Blood was taken twice from the vein: before the first and after the last injections. The significance of differences was estimated by Student's test for small samples. The animals were killed 1 h after the last injection of the preparations and fragments of the removed lungs and kidneys were fixed in a 10% solution of neutral formalin and embedded in paraffin wax. Sections were stained with hematoxylin and eosin and also for fibrin by Shueninov's method.

EXPERIMENTAL RESULTS

In the animals of group 1 alternation of emphysematous foci with regions of multiple atelectasis was found. The alveolar septa were thickened, with marked edema and intensive cellular infiltration, which also were discovered around the bronchi, arteries, and veins of varied caliber (Fig. 1a, b). Vessels of average and small caliber were in a state of spasm, justamural mixed and red thrombi were discovered in them, and in some vessels there was hemolysis of erythrocytes. In many preparations a phenomenon characteristic of the action of PAF was observed in the lung tissue: pavementing of leukocytes and the outflow of these cells into the perivascular space, which was connected with the powerful chemotaxic action of the mediator.

An increase in the number of cells in the stroma was found in the kidney tissues, similar in appearance to cellular infiltration of the lung, with interstitial edema and cloudy-swelling degeneration of the epithelium of the tubules with dilatation of their lumen (Fig. 1c, d). In the mesangitm of the glomeruli the number of cells was also increased, mainly due to infiltration with mononuclears and proliferation of mesangiocytes. Vascular spasm was distinctly visible. In vessels of medium caliber and in the microcirculatory bed in which spasm was present, the formation of juxtamural thrombi could be seen. Deposition of a network of fibrin was observed along the inner surface of the wall of the vessels and in the mesangium of many glomeruli. The plasma fibrinogen level in the animals of group 1 was significantly elevated (571 ± 47 mg% compared with 395 ± 41 mg% in the control group, $p < 0.05$).

Foci of dystelectasis and atelectasis, edema, and cellular infiltration of the alveolar septa also were observed in the lung tissue of the animals of group 2 (Fig. 2a), but signs of spasm were absent in most pulmonary vessels, and juxtamural thrombi and fibrin deposits were found only in some of them.

In the kidney tissues of animals receiving PAF and verapamil, cellular infiltration, interstitial edema, and degeneration of the tubules also were found. However, thrombi and fibrin deposits were virtually absent in the vessels (Fig. 2b). Moreover, despite the marked vasoactive effect, as shown by pycnosis of the endothelium, no vessels in spasm could be observed (Fig. 2c). Elevation of the plasma fibrinogen level in these animals was not significant compared with the control ($p > 0.05$).

Thus repeated injections of PAF in increasing doses caused disturbances of the microcirculation, thrombosis, and an infiltrative inflammatory reaction in the lungs and kidneys resembling interstitial nephritis, pneumonitis, and vasculitis. Edema and microcirculatory disturbances, highly characteristic of shock, especially endotoxin shock, for which FAF is a very important mediator [3], also were present. With the mode of administration used in this investigation, PAF can pass through the lungs and act on the second passage directly on the kidneys. Under these circumstances it induces chemotaxis of mono- and polynuclears, which give cellular infiltration of the organs and also activation and aggregation of platelets and neutrophils, followed by the release of secondary mediators and blood clotting factors, deposition of fibrin, and thrombus formation. Verapamil effectively blocks vascular spasm, leads to a fall in the plasma fibrinogen level, and prevents fibrin deposition and thrombus formation. This confirms the calcium-dependent mechanism of the vasospasm and thrombogenesis induced by PAF in vivo and it is in agreement with data in [7]. Meanwhile verapamil, in a dose of 0.5 mg/kg, caused hardly any change in the intensity of cellular infiltration and did not depress the manifestation of edema in the target organs, possible evidence of the diversity of the mechanisms realizing the broad spectrum of action of PAF in vivo.

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