CHANGES IN THE MICROCIRCULATION DURING THE COMBINED ACTION OF Clostridium perfringens TYPE A TOXIN AND METABOLIC PRODUCTS OF Clostridium butyricum

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UDC 612.822.3.014.46:[576.851.555.097.29]-06:615.214.22

In poisoning caused by injection of Clostridium perfringens type A toxin or a mixture of toxin with the filtrate of a culture of Clostridium butyricum, changes in the microcirculation in the mucous membrane of the retrobuccal pouch of golden hamsters were found. The microcirculatory changes took place in two phases. In the first phase vasomotor disturbances were observed, as shown by periodic changes in diameter of the arterial microvessels, and by plasmatization and a decrease in the number of functioning capillaries. The diameter of the veins showed no significant change. The second phase was characterized by persistent disturbances of the microcirculation: slowing of the blood flow in the arterial and venous portions, the appearance of regions of stasis, a retrograde blood flow, and arterial dilatation. The persistent disturbances of the microcirculation following injection of a mixture of Cl. perfringens toxin and filtrate of a broth culture of Cl. butyricum appeared sooner than those in response to injection of the toxin alone.

KEY WORDS: Clostridium perfringens; Clostridium butyricum; microcirculation.

The clinical and pathophysiological features of diseases caused by anaerobic microorganisms and, in particular, by Clostridium perfringens suggest that disturbances of the microcirculation are an important link in the pathogenesis of these toxicoinfections. However, only isolated studies of this problem have been undertaken [3, 8, 9, 15]. In anaerobic gas-gangrene infection associant microorganisms play an important role [1, 2, 4, 13]. Metabolic products of one of these associants, namely Clostridium butyricum which themselves have no toxic action, have been shown to potentiate the effects of Cl. perfringens type A toxin [6, 7].

The object of this investigation was to study changes in the microcirculation in poisoning due to Cl. perfringens type A toxin and metabolic products of Cl. butyricum.

EXPERIMENTAL METHOD

Dried Cl. perfringens type A toxin, of batch 29, manufactured by the Khar'kov Research Institute of Vaccines and Sera (filtrate of a broth culture) and Cl. butyricum strain 253 were used. Before the experiment, 100 ml of the lyophilized filtrate was dissolved in distilled water and injected into animals in a dose of 0.2 ml of filtrate/100 g body weight. The state of the microcirculation was observed in an apparatus for biomicroscopy, mounted on the base of a MBI-3 microscope, in the mucous membrane of the retrobuccal pouch of a hamster kept in a transparent plastic container [5, 14]. The animal was anesthetized with pentobarbital. Photographic recording was carried out with the MFN-II photomicrographic attachment on "Mikrat-300" film. Experiments were carried out on 15 golden hamsters weighing 130-160 g: Six hamsters were given an intraperitoneal injection of 1 LD of Cl. perfringens toxin in 1 ml physiological saline (group 1), five animals received a mixture of 1 LD toxin and filtrate of a broth culture of Cl. butyricum in a volume of 1 ml (group 2), and four hamsters received the filtrate of the broth culture of Cl. butyricum only (control).

Department of Microbiology, N. I. Pirogov Odessa Medical Institute. Laboratory of Pathophysiology of Extremal States, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. N. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 11, pp. 552-554, November, 1977. Original article submitted March 23, 1977.

0007-4888/77/8411-1571 $07.50

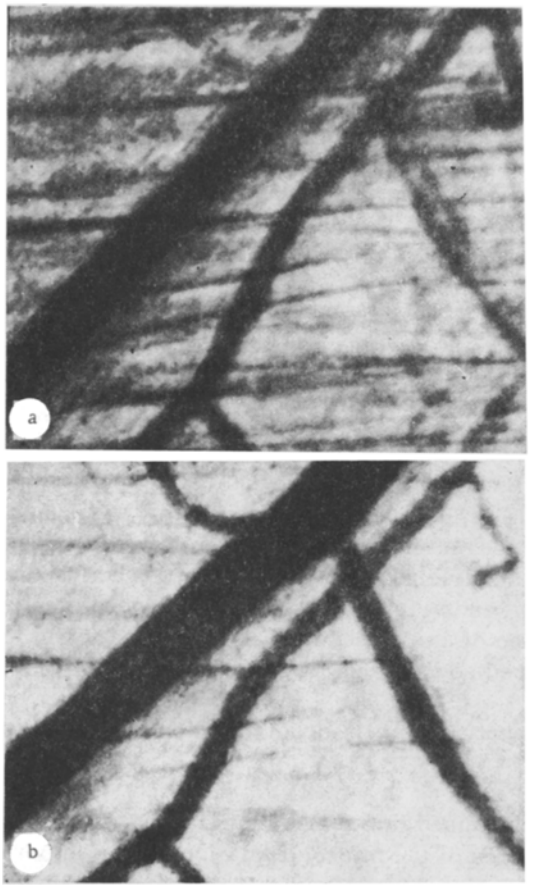

Fig. 1

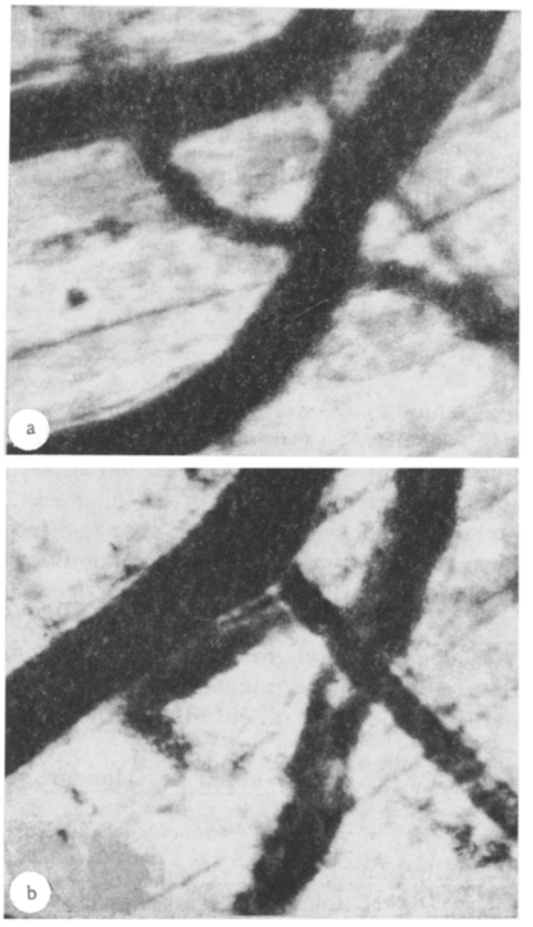

Fig. 2

Fig. 1. Microvessels of mucous membrane of retrobuccal pouch of a hamster before injection of Cl. perfringens type A toxin (a) and 5 h 10 min after its injection (b). Magnification 60×. Explanation in text.

Fig. 2. Microvessels of mucous membrane of hamster retrobuccal pouch before simultaneous injection of Cl. perfringens type A toxin and filtrate of broth culture of Cl. butyricum (a) and 4 h after their injection (b). Magnification 60×, explanation in text.

EXPERIMENTAL RESULTS AND DISCUSSION

In the animals of group 1, increased vasomotor activity was observed 4-5 h after injection of the toxin, as shown by periodic changes in the diameter of the arterial microvessels, which were temporarily constricted either along their whole length or segmentally. As a result of sharp constriction of the arteries and also, probably, of the precapillary sphincters plasmatization of some arterial microvessels and capillaries took place and the number of functioning capillaries was reduced. The diameter of the veins showed no significant change: The blood only flowed more slowly in them or stasis developed in the smallest vessels. The changes described were transient and after a short time the constriction of the arterial microvessels was replaced by restoration of their original diameter, the plasmatized vessels were filled with blood cells, and the blood began to flow again in the smallest venules and capillaries. Permanent impairment of the microcirculation occurred after 4-5 h or more. Slowing of the blood flow in the arterial and venous portions of the microcirculation was observed, the number of functioning capillaries was reduced, and regions of stasis appeared. A to-and-fro or retrograde blood flow was recorded at this time. As a rule dilatation of the arterial vessels was found, their diameter being increased by 1.5-2 times (Fig. 1). In the flow of red cells in the arteries, groups of adherent leukocytes were observed to move. In the arterial and venous vessels of all the animals there were small, unstable aggregates of red cells. The changes in the diameter of the veins were inconstant. The leukocytes in the veins were arranged at the periphery. Four of the animals died during the first day after injection of the toxin and two at the end of the second day.

In the animals of group 2 the lasting disturbances of the microcirculation were similar in character to

those in the animals of the previous group, but they appeared sooner. As early as after 3-4 h a persistent slowing of the blood flow in the arterial and venous portions of the microcirculation was observed, the number of functioning capillaries was reduced, and regions of stasis appeared; in some vessels a to-and-fro or retrograde flow was observed. Dilatation of the arteries was recorded, their lumen being enlarged by 1.5-3 times (Fig. 2). Small aggregates of leukocytes were observed in the arterial blood flow. There were no sharp changes in the diameter of the veins, but the leukocytes in them were arranged peripherally. Death of all the animals occurred during the first day after injection of the toxin and filtrate.

In the animals of the control group, which received only filtrate of Cl. butyricum, no gross disturbances of the microcirculation were found.

The results indicate that Cl. perfringens type A toxin, alone or together with the filtrate of a broth culture of Cl. butyricum, causes definite changes in the microcirculation. Vasomotor disturbances were observed for a long period of time (4-5 h) after injection of the toxin. Kozlov and Ispolatovskaya [3] observed periodic wave-like contraction of the muscular components of the small arteries and arterioles, followed by their relaxation, 3-5 min after injection of Cl. perfringens type A toxin. A persistent constriction of the arteries developed after 0.5-1 h, whereas the veins were dilated and distended with blood. These workers associate this phenomenon with a direct disturbance of the hemodynamic function of the microcirculation as a result of the action of the toxin. In the present experiments the vasomotor phenomena lasted much longer. The lumen of the veins at these times showed no significant change. In the experiments with the combined injection of Cl. perfringens toxin and filtrate of a broth culture of Cl. butyricum vasomotor disturbances of the same type were observed, but they lasted not more than 3-4 h, after which the persistent changes arose. In the experiments in which the filtrate of Cl. butyricum alone was given, the intensity of the vasomotor phenomena was low.

Increased vasomotor activity was evidently not a specific manifestation of the action of Cl. perfringens toxin, for it is observed in response to the action of various factors, notably in hemorrhagic [11] and traumatic shock [10]. A lasting disturbance of the microcirculation was observed 4-5 h after injection of Cl. perfringens toxin and 3-4 h after combined injection of Cl. perfringens toxin and Cl. butyricum filtrate. The blood flow in the arterial and venous portions of the microcirculation was slowed, the number of functioning capillaries was reduced, regions of stasis appeared, and the arteries were sharply dilated.

The sharp dilatation of the arteries and the less marked response of the veins observed in these experiments may indicate that Cl. perfringens toxin has a greater action on the walls of the arterial than of the venous microvessels. In endotoxin shock [12], the capacitive vessels also preserve their tone longer than the resistive vessels.

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