TIME COURSE OF CARREGEENAN-INDUCED INFLAMMATION DURING EXPERIMENTAL THERAPY

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UDC 615.356:577.164.3].
036.8:616-002-092.9-08

KEY WORDS: inflammation; microcirculation; carrageenan; rutin; aesculamine.

Among drugs with vitamin P activity there are some with a marked anti-inflammatory action [7]. One of the important criteria of a promising anti-inflammatory agent is that it should inhibit the development of carrageenan-induced inflammation. It was shown previously [5, 6] that a substance with vitamin P activity, namely the water-soluble hydrochloride of 8ββ'-dihydroxydiethylaminomethyl-4-methylaesculetin (aesculamine), synthesized in the Research Institute of Pharmacology, Academy of Medical Sciences of the USSR [2], if injected beforehand, accelerates restoration of the microcirculation after carrageenan-induced disorders.

In the investigation described below the time course of inflammation during administration of rutin and aesculamine after injection of carrageenan was studied.

EXPERIMENTAL METHOD

The following models of carrageenan inflammation were used: 1) The pathological process developing in the hamster retrobuccal pouch in response to application of 1 ml of an aqueous suspension of 0.5 mg carrageenan to its mucosa; 2) edema developing in the hind limb of a rat after subplantar injection of 1 ml of an aqueous suspension of 0.5 mg carrageenan. Distilled water (1 ml) was injected into the control animals. Biomicroscopy of the mucosa of the hamsters' retrobuccal pouch was carried out daily for 5 days, using the transparent chamber method [4]. The external diameter of the arterioles (40-45 μ) and venules (50-55 μ) was measured by the image splitting method [1] on the 3rd day after application of carrageenan [1], for that is the time when the most marked disturbances of the microcirculation develops [5]. In the other model of inflammation the extent of tissue edema of the rat's hind limbs was measured by means of a measuring cylinder, and the skin temperature of the limbs was recorded with TPÉM-1 electrothermometer, and their pain sensitivity was measured with an "Analgesia Meter for the Rat Paw" (Italy). The measurements were made 1 and 3 h after injection of carrageenan, at the height of the first and second phases of inflammation [7]. Experiments were carried out on 48 male golden hamsters weighing 200 g and on 48 male Wistar rats weighing 120 g. In all the experiments rutin and aesculamine were injected subcutaneously in the dorsal region in a dose of 50 mg/kg body weight 30 min after administration of the inflammogen. The results were subjected to statistical analysis [3].

EXPERIMENTAL RESULTS

Microcirculatory disorders were observed 24 h after application of carrageenan to the mucosa of the hamster retrobuccal pouch: Tissue edema, slowing of the blood flow, and diapedesis of erythrocytes and leukocytes, evidence of the beginning of an acute inflammatory process [7]. On the 3rd day the diameter of the arterioles and venules was considerably increased compared with the control (Table 1). By the 5th day the inflammatory process had subsided. Rutin did not affect this model of carrageenan inflammation: the diameter of the microvessels on the 3rd day and the microcirculatory changes were the same as in the experiment

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0007-4888/86/0002-0167$12.50

TABLE 1. External Diameter of Microvessels of Mucosa of Hamster Retrobuccal Pouch on 3rd Day after Preliminary Application of Carrageenan and Treatment with Rutin and Aesculamine (M ± m)

Experimental conditions	Number of animals	Diameter of arterioles	Diameter of venules
		μ	
Control (distilled water)	12	40,5±0,18	52,1±0,13
Carrageenan	12	54,8±0,12*	68,8±0,19*
Carrageenan + rutin	12	52,1±0,17*	68,4±0,15*
Carrageenan + aesculamine	12	42,0±0,15	53,2±0,19

Legend. *P < 0.05 compared with control. Differences between series "Carrageenan" and "Carrageenan + rutin" is not significant (P > 0.05).

TABLE 2. Volume, Temperature, and Pain Sensitivity of Rat Hind Limbs after Subplantar Injection of Carrageenan and Treatment with Rutin and Aesculamine (M ± m)

Time after injection of carrageenan	Experimental conditions	Number of measurements	Volume of paw, ml^3	Skin temperature, °C	Pain threshold, g
1 h	Control (distilled water)	12	1,1±0,13*	24,6±0,21*	327,5±20,3*
	Carrageenan	12	2,7±0,15	35,2±0,70	187,6±31,6
	Carrageenan + rutin	12	1,2±0,18*	25,8±0,15*	339,6±36,7*
	Carrageenan + aesculamine	12	0,9±0,13*	26,0±0,32*	341,8±41,0*
3 h	Control (distilled water)	12	1,3±0,10*	26,2±0,41*	338,2±39,1*
	Carrageenan	12	3,0±0,18	34,8±0,13	125,9±27,8
	Carrageenan + rutin	12	2,9±0,14	35,0±0,67	131,8±32,2
	Carrageenan + aesculamine	12	1,2±0,11*	25,8±0,21*	325,9±40,1*

Legend. *P < 0.05 compared with injection of carrageenan.

with application of carrageenan but without treatment. Meanwhile aesculamine abolished the inflammatory reaction: the diameter of the arterioles and venules was not increased compared with the control on the 3rd day. Microcirculatory disturbances described above also were absent.

The results of the experiments on rats showed that subplantar injection of carrageenan evoked a biphasic inflammatory reaction in the tissues of the rat's paw (Table 2). The volume of the paw and pain sensitivity were increased and the skin temperature raised 1 h (first phase) and 3 h (second phase) after injection of carrageenan. Rutin and aesculamine inhibited development of the first phase of inflammation. Rutin did not affect the development of the second phase, whereas this phase did not arise after injection of aesculamine.

This investigation thus showed for the first time that aesculamine, unlike rutin, when given therapeutically, inhibits inflammation induced by administration of carrageenan. Edema of the rat limb is known [9] to be connected with release of histamine and serotonin from depots for the first hour, but after 3 h it is due to synthesis of prostaglandins in the inflammatory focus. These results suggest that when rutin and aesculamine are used therapeutically, their anti-inflammatory activity at the beginning of the inflammatory process is associated with their ability to inhibit release of histamine and serotonin. Unlike rutin, aesculamine evidently blocks prostaglandin synthesis in the later stages of the inflammatory process induced by carrageenan, and this explains its therapeutic activity. Consequently, aesculamine may be a promising anti-inflammatory agent not only in the early, but also in the late stages of acute inflammation.

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ROLE OF LEUKOTRIENES IN SHOCK OF IMMUNE GENESIS

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UDC 616-001.36-056.43-092.9-07:
616.1-008.1-02:615.31:547.295.96

KEY WORDS: leukotrienes; shock; cardiac anaphylaxis.

The writer showed previously that immune damage to the heart is accompanied by a shock reaction, due to disturbance of the contractile function of the myocardium and mediated humorally through stored blood [2-5].

One possible humoral intermediary in the development of disturbances of the cardiodynamics and hemodynamics of immune genesis may be a slow reacting substance A (SRSA) — a vasoactive mediator of hypersensitivity reactions of immediate type [1, 6, 14]. It has recently been shown that SRSA consists of leukotrienes C_4, D_4, and E_4 — representatives of a new class of biologically active substances, which are derivatives of arachidonic acid by the lipoxygenase metabolic pathway [11, 13]. Leukotrienes are formed and secreted from the heart in considerable amounts during cardiac anaphylaxis [10]. When introduced into the body they can cause considerable disturbances of the cardio- and hemodynamics [12], with the development of coronary spasm, focal ischemia, and contractile failure of the myocardium [8, 9].

To determine the role of leukotrienes in the development of disturbances of the cardio- and hemodynamics associated with cardiac lesions of immune genesis, in the investigation described below the response of the circulatory system to immune trauma of the heart, with and without blockade of leukotriene biosynthesis was compared *in vivo*.

EXPERIMENTAL METHOD

Acute experiments were carried out on mongrel dogs weighing 17-22 kg under chloralose-urethane anesthesia (0.05 and 0.3 g/kg, respectively). There were two series of experiments: I) Control, in which changes in the hemodynamics and cardiodynamics were studied in intact dogs subjected to immune trauma to the heart, and II) experimental, in which changes in the same parameters under similar conditions were studied in dogs after preliminary blockade of lipoxygenase, i.e., of leukotriene biosynthesis. Lipoxygenase was blocked by means of quercetin, a solution of which was injected intravenously in a dose of 10 mg/kg [15]. Immune trauma to the myocardium of the left ventricle was inflicted 10-15 min after injection of quercetin, and changes in the cardio- and hemodynamics were observed for 1 h. The heart was traumatized by injection of 1 ml of a solution of immune anticardiac γ-globulin (1 mg/kg) into one branch of the left coronary artery, without opening the chest.

The globulin was obtained from an anticardiac serum with anticardiac antibody titer in the complement fixation test of 1:320-1:640.

The following parameters of the cardio- and hemodynamics were recorded in the experiments: The systemic blood pressure (BP), the central venous pressure (CVP), the perfusion pressure

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Department of Experimental Cardiology, A. A. Bogomolets Institute of Physiology, Academy of Sciences of the Ukrainian SSR. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Gorev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 2, pp. 151-153, February, 1986. Original article submitted March 6, 1985.

