ACTION OF DEFENSIN ON HEALING OF ASEPTIC SKIN WOUNDS AND ON VASCULAR PERMEABILITY

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UDC 616.5-001.4-021.4-092.9-085.28 -036.8:616.5-003.9

Key words: defensin; aseptic wounds; vascular permeability.

Defensins (D) are cationic low-molecular-weight (under 5000 daltons) nonenzymic proteins, isolated from lysosomes of macrophages and neutrophils [9, 10]. The principal functional property of these proteins is to protect man and animals against bacterial, fungal, or viral agents of infectious diseases [5, 9, 10]. The D molecule is characterized by alternation of cationic and hydrophobic regions, which makes this peptide a powerful membranotropic agent [9, 10]. It has been found that D increase the permeability of the vascular wall and stimulate growth of microvessels [3, 8]. Leukocytic serum, used in the treatment of open fractures, of postoperative wounds, and of other inflammatory conditions is known to contain degradation products of neutrophils [4]. They may perhaps also contain D. The facts described above suggest that administration of D in the peptide form may have a beneficial effect on repair processes, including on the healing of skin wounds.

The aim of this investigation was to study the effect of repeated intramuscular injections of D on the healing of a full-thickness skin wound under aseptic conditions; the action of D on vascular permeability was investigated by qualitative and semiquantitative methods.

EXPERIMENTAL METHOD

D were obtained from the peritoneal exudate of rabbits. Experiments were carried out on 26 male albino rats weighing 200-240 g. The 13 experimental animals received daily injections of D in a dose of 125 μg/kg body weight, dissolved in 0.3 ml physiological saline. The 13 control rats each received 0.3 ml of physiological saline. The injections were given into the gastrocnemius muscle of the animal's hind limb 2 days before wounding, and they continued until the 14th day after the operation. The full thickness skin wound was inflicted with a pointed object in the region of the spine a little to the right of the vertebral column, and the wound was covered with collodion. The length of the wound and its site were always constant, the initial length of the wound being 10 mm. The rate of repair was judged by changes in the length of the wound on the 2nd, 5th, 7th, and 13th-15th days after the operation. Vascular permeability was studied with the aid of trypan blue and colloidal carbon. For quantitative determination of vascular permeability the rats were bound in the supine position, the abdomen was shaved, and trypan blue was injected intravenously in a dose of 15 mg/kg. After 4-10 min 0.1 ml of D (125 μg/kg) and the solvent (physiological saline) were injected intradermally. Thus two papules were formed on the abdominal skin of each rat — an experimental one with D and a control with physiological saline. The area of the blue part of the papule was measured 10 min later, disregarding the blue staining at the site of puncture. Ten rats were used for the experiments. For the semiquantitative method, colloidal carbon was obtained from ink. The ink was evaporated down for 50 min at 60°C and the supernatant was centrifuged for 1 h at 8000 rpm. Particles of colloidal carbon thus obtained measured 20-50 nm in diameter and are deposited around the perimeter of the endothelial cells of blood vessels [7]. Between 5 and 10 min after intraperitoneal injection of D (125 μg/kg) or physiological saline into the rats, they were given an intravenous injection of this colloidal carbon in a dose of 0.2 ml/100 g body weight. Ten rats received the peptide and 10 received physiological saline. The animals were killed 30 min after injection of colloidal carbon

Department of Human and Animal Physiology, Faculty of Biology, Moscow University. Laboratory, Research Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 4, pp. 391-393, April, 1990. Original article submitted June 28, 1989.

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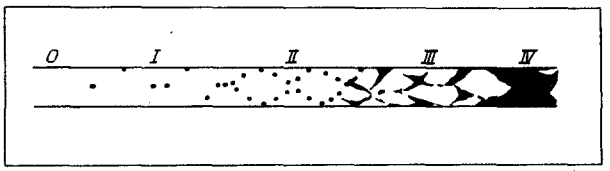

Fig. 1. Diagram showing degrees of disturbance of vascular permeability, according to M. P. Gorizontova [1]. I) Diffuse (dust-like) dot labeling; II) structured (punctiform) dot labeling; III) patchy labeling; IV) confluent (muff-like) patchy labeling.

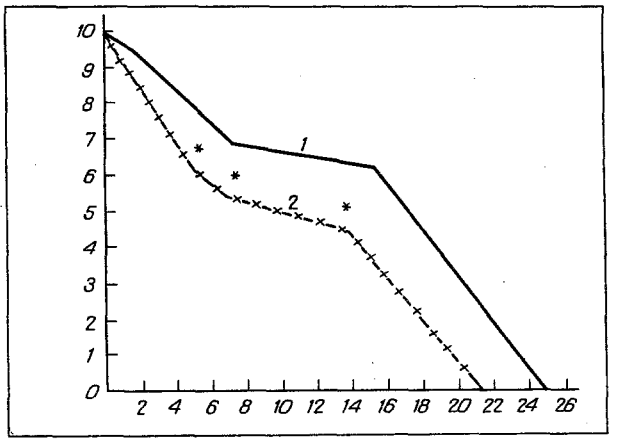

Fig. 2. Time course of healing of full-thickness aseptic skin wound in control (1) and in animals receiving intramuscular injections of D (2). Abscissa, days after operation; ordinate, length of wound (in mm). Asterisks indicate days after operation on which a significant ($p < 0.01$) difference was found between control and experiment.

and the mesentery was excised and laid flat on a slide. Disturbances of vascular permeability were judged by the number of labeled vessels in 10 mesenteric windows examined, and the intensity of the label. A conventional scale of label intensity from 0 to IV is illustrated schematically in Fig. 1 [1].

EXPERIMENTAL RESULTS

Examination of the graphs in Fig. 2 shows that starting with the 5th day the process of wound healing took place faster in the animals receiving injections of D than in rats receiving physiological saline ($p < 0.01$). On the 5th day the length of the wound in the experimental animals was reduced to 59.2 ± 1.7%, compared with 77.1 ± 1.2% in the control animals (the initial length of the wound is taken as 100%). On the 7th day after the operation the length of the wound in the experimental rats also was less than in the controls, namely 54.2 ±0.8% and 68.5 ± 1.3%, respectively. The length of the wound, measured on the 13th-15th day, was as before less in the rats receiving D (45.3 ± 1.9%) compared with the control rats (59.7 ± 2.1%). The scab separated sooner in the experimental group of rats, namely on the 9th-11th day, compared with the 13th-15th day in the control rats. Wound healing was complete in the control rats on the 24th-26th day after the operation, but in the animals receiving D injections it was complete on the 21st-22nd day.

Thus with the aid of this model of a full-thickness skin wound rapid regeneration of the tissues could be detected in rats receiving D. The time course of the cellular reactions in the focus of aseptic inflammation is sufficiently familiar. Active proliferation of cells in the zone of aseptic inflammation is observed on the 4th-5th day [6]. It can be tentatively suggested that under the influence of D the intensity of proliferation is increased. In the experiments with trypan blue the papule with physiological saline did not stain whereas the papule with D became blue.

TABLE 1. Vascular Permeability under the Influence of D

Experimental conditions	Number of mesenteric windows (in % of total number examined)			Number of rats with predominance of the undermentioned degree of labeling (in % of total number of rats in expt.)				
	without label	1-10 labeled vessels	over 10 labeled vessels	0	I	II	III	IV
Physiological saline	97	2	1	80	20	0	0	0
Defensins	30	30	40	10	20	20	40	10

It will be clear from Table 1 that D disturb vascular permeability. In 70 of 100 mesenteric windows examined from rats receiving D, vessels labeled with colloidal carbon could be seen. In each such rat the whole range of degrees of labeling was observed, but usually one degree of labeling predominated. In most experimental rats III degree (patchy labeling) predominated. Only three of the 100 mesenteric windows of the control rats examined had labeled vessels, and the I degree, namely diffuse dust-like labeling, was observed in only two animals.

Thus defensins increase vascular permeability, in agreement with data obtained in the West [8]. The morphological substrate for increased microvascular permeability is composed of relatively wide transmural channels, formed temporarily in the zone of contact between endotheliocytes as a result of their increased mobility [2]. During long-term adaptation to the action of defensins disturbing contacts between epithelial cells, the proliferative activity of these cells is probably increased, and new capillaries are formed [3], whereas in the case of disturbance of the integrity of the skin, surviving cells around the edges of the wound slide into it.

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