EFFECT OF COLLAGEN-DALARGIN COMPLEX ON BLOOD FLOW IN SKIN WOUND TISSUES AND ON SYSTEMIC HEMODYNAMICS

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In recent years, through the intensive study of the biological properties of peptides, data have been obtained on a new pharmacologic effect of the opioid peptides, namely their ability to stimulate regeneration of many tissues [1, 5-7, 15]. The universal nature of the reparative response of different organs to opioid ligands, on the one hand, confirms the previous hypothesis concerning their role in the regulation of structural homeostasis [2, 3], and on the other hand it suggests the existence of a dominant stage, a single mechanism lying at the basis of repair. The chief indicator of activity of the healing process is the intensity of capillary formation at sites of injury [12]. As a rule healing takes place more rapidly in better vascularized tissues than in those less well vascularized [10].

The aim of this investigation was to study the effect of dalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg), a structural analog of Leu-enkephalin with opioid properties, in the form of a complex with collagen, on the capillary blood flow in various parts of full-thickness skin wound in the dorsal region of rats and parameters of the systemic hemodynamics in the animals at different times during wound healing.

EXPERIMENTAL METHOD

Experiments were carried out on 23 male Wistar albino rats weighing initially 260-300 g. The animals were divided into three groups: 1) control, 2) the wounds on the rats were covered with collagen sponge, 3) the wounds on the rats were covered with collagen-dalargin complex at the rate of $2.4 \cdot 10^{-5}$ g dalargin/kg body weight. Parameters of the regional and systemic hemodynamics were assessed by the use of radioactive microspheres [9, 11] in the modification in [4]. Under pentobarbital anesthesia, PE10 polyethylene catheters (Portex, England) were introduced into the abdominal aorta through the femoral artery and into the left ventricle through the right carotid artery, and the ends of the catheters were brought up through a subcutaneous tunnel and fixed to the skin in the interscapular region. After 24 h a full-thickness skin flap 22 mm in diameter was removed under ether anesthesia from a particular region of the animal's back. To prevent contraction, Teflon rings of suitable diameter were sutured into the wound edges, and covered with lids for fixation of the collagen and to prevent the possibility of infection. The animals were kept in individual cages. The sponge was changed once a day for 7 days. The excess of exudate was removed from the control animals with sterile towels. The rules of asepsis were observed during all operations. The blood pressure (BP) and heart rate (HR) were recorded in the aorta (Hellige, West Germany) of the unrestrained rats on the 3rd, 5th, 7th, and 10th days. At the same times a suspension of radioactive microspheres 15 μ in diameter, washed to remove dextran and labeled with ^{141}Ce, ^{51}Cr, ^{95}Nb, or ^{46}Sc (New England Nuclear, USA), was injected at these same times into the left ventricle. About 100,000 microspheres were injected in a single dose. After the last injection the animals were killed with an overdose of pentobarbital and frozen at −20°C to facilitate dissection of the tissues. Regions of the regenerating wound (Fig. 1) and also of internal organs and intact skin were weighed and the number of microspheres taken up was determined on a CompuGamma model 1281 gamma-counter (LKB, Sweden). All calculations were done

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TABLE 1. Dynamics of Blood Flow (in ml/min·g) in Different Parts of Regenerating Skin Wounds under the Influence of Collagen-Dalargin Complex (M ± m)

Test object	Time of investigation, days	Control (8)	Collagen (7)	Collagen-dalargin (8)
Granulation tissue in floor of wound	3	1,08±0,19	1,55±0,21	3,18±0,36 a,b
	5	2,16±0,24	3,44±0,47 a	1,86±0,24 b
	7	2,37±0,34	4,27±0,80 a	1,42±0,26 a,b
	10	1,25±0,28	2,31±0,61	1,14±0,20
Muscle tissue beneath granulation tissue	3	0,91±0,11	0,94±0,14	1,20±0,12
	5	0,78±0,12	0,54±0,09	1,04±0,21
	7	0,74±0,13	0,47±0,10	0,84±0,25
	10	0,64±0,10	0,55±0,08	0,52±0,05
Muscle fascia adjacent to wound	3	—	0,70±0,16	0,75±0,12
	5	—	0,83±0,11	0,95±0,18
	7	—	0,85±0,35	0,65±0,15
	10	—	0,74±0,15	0,70±0,11
Muscle tissue adjacent to wound	3	—	0,56±0,05	0,45±0,04
	5	—	0,41±0,06	0,44±0,06
	7	—	0,35±0,05	0,38±0,03
	10	—	0,43±0,05	0,32±0,05
Area of skin adjacent to wound	3	0,54±0,09	0,47±0,04	0,55±0,08
	5	0,52±0,06	0,51±0,08	0,48±0,05
	7	0,46±0,04	0,51±0,07	0,50±0,07
	10	0,40±0,05	0,62±0,11	0,59±0,04
Intact dorsal skin	3	0,35±0,06	0,41±0,06	0,40±0,05
	5	0,35±0,05	0,39±0,07	0,38±0,04
	7	0,30±0,03	0,33±0,04	0,33±0,04
	10	0,20±0,02	0,37±0,06 a	0,37±0,03 a

Legend. Here and in Table 2: number of animals shown in parentheses; a) $p < 0.05$ compared with control, b) $p < 0.05$ compared with animals treated with collagen.

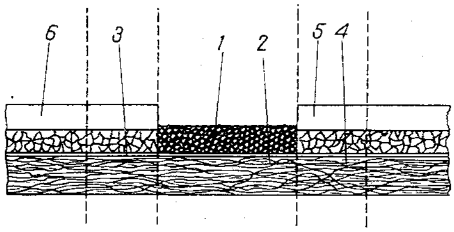

Fig. 1. Scheme showing regions of dorsal skin wound studied in rats. 1) Granulation tissue of wound floor; 2) muscle tissue beneath granulation tissue; 3) muscle fascia adjacent to wound; 4) muscle tissue adjacent to wound; 5) area of skin adjacent to wound; 6) intact forsal skin.

by the standard equations [11] on a Labtam 3015 computer. Dalargin was synthesized in the Laboratory of Peptide Synthesis, Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, and the collagen-dalargin complex was prepared at the Central Research Laboratory of the I. M. Sechenov First Moscow Medical Institute. The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The blood flow in the granulation tissue of the untreated animals reached a maximum on the 7th day (Table 1), in full agreement with results obtained on an analogous open wound model in rats [13]. This period corresponds to the formation of granulation tissue with vertical vascular loops [8]. Later, during maturation of the granulation tissue, the number of capillaries decreased and the blood flow was reduced. Under the influence of collagen, on the 5th day after the operation a significant increase in blood flow was observed in the granulation tissue compared with the untreated wound, and just as in the control, it reached a maximum on the 7th day, although this was almost twice as high as the control value. The blood flow was reduced somewhat on the 10th day, on maturation of the granulation tissue. It is thus clear that although collagen intensifies the blood flow, its effect is manifested only during the natural cycle of capillary formation in the granulating wound. Possibly this

TABLE 2. Parameters of Systemic Hemodynamics in Rats during Treatment of Wounds with Collagen-Dalargin Complex

Parameter of hemodynamics	Time of investigation, days	Control (8)	Collagen (7)	Collagen-dalargin (8)
BP, mm Hg	3	94,3±2,7	85,5±1,5[a]	84,7±1,7[a]
	5	89,5±2,7	85,2±1,6	82,7±1,5
	7	85,7±2,0	89,7±3,7	82,1±1,3
	10	88,3±2,1	87,8±2,2	83,0±1,8
HR, beats/min	3	480,8±12,4	440,8±4,7[a]	430,1±11,3[a]
	5	451,1±12,7	426,2±6,2	413,0±10,0[a]
	7	448,1±7,2	428,7±9,2	413,1±9,6[a]
	10	425,3±11,0	414,5±5,3	424,3±7,5
Cardiac output, ml/min	3	136,4±6,4	144,1±5,8	143,8±6,8
	5	142,7±8,7	142,2±7,8	147,3±6,9
	7	162,0±7,1	156,9±6,9	146,8±9,4
	10	141,8±9,0	157,2±4,9	149,9±8,0
Cardiac index (CI), ml/min·100 g	3	49,7±2,3	51,3±1,7	48,4±2,0
	5	53,0±2,8	51,9±2,9	50,2±1,7
	7	60,6±3,0	57,0±2,6	50,0±2,2[a]
	10	54,4±4,0	57,0±1,4	51,2±1,8[b]
Total peripheral resistance (BP/CI)	3	1,92±0,08	1,68±0,07	1,78±0,10
	5	1,72±0,09	1,68±0,11	1,67±0,08
	7	1,43±0,05	1,57±0,09	1,67±0,07[a]
	10	1,67±0,10	1,54±0,05	1,64±0,07

response reflects the property of the collagen sponge not only to stimulate fibroblast function, but also to absorb the excess of exudate, containing serum proteins, prostaglandins, fibrinolytic factors, etc., like the effect of hydrophilic wound covering this which, in the opinion of some workers [14], reduces the intensity of components of the inflammatory reaction and promotes collagen formation and stimulates the blood flow.

Application of collagen-dalargin sponge to the wound surface led to a marked increase in the capillary blood flow in the granulation tissue as early as on the 3rd day after the operation, i.e., 4 days sooner than in the control animals, corresponding to the earlier formation and, consequently maturation, of the capillaries. This is shown by reduction of the blood flow on the 5th day compared with the 3rd and a significant reduction on the 7th day compared with untreated wounds and wounds treated with collagen. These findings are confirmed by the results of a histologic and histochemical study of granulating wound tissues under the influence of dalargin [8], which demonstrate the more intensive proliferation of fibroblasts on the 2nd-4th day with an increase in the number of mitoses, their rapid differentiation, and potentiation of the secretion of acid glycosaminoglycans and collagen, stimulation of collagen fibrillogenesis, and the earlier and more active growth of vascular elements, namely endothelial bands and capillaries.

Similar changes in the blood flow were observed also in other parts of the skin wound in the course of healing and, in particular, in the muscle tissue beneath the layer of granulation. The intensity of the blood flow in undamaged tissues, however, was unchanged as a result of treatment with dalargin (Table 1).

Under the influence of the operation, the animals developed some degree of tachycardia in the early stages, with an increase in cardiac output (CO) and cardiac index (CI) toward the 7th day after the operation (Table 2). Local application of collagen-dalargin sponge had a definite stabilizing effect on all these parameters: the mean values of BP, HR, CO, and CI remained virtually constant during wound healing. It can accordingly be concluded that changes observed in the regional blood flow in the tissues of a regenerating wound following application of dalargin are not connected with its effect on parameters of the systemic hemodynamics.

The data given above are evidence that at least one component of the mechanism of the effect of dalargin as a stimulator of repair of damaged tissues may be activation of the blood flow in the regenerating tissues. The blood flow in granulation tissue is a function of the degree of vasodilatation and of the number of functioning blood vessels [13]. Analysis of results obtained by the radioactive microspheres method and by histologic and histochemical methods indicates that both mechanisms are involved in stimulation of the blood flow in an open wound during the early period of healing under the influence of dalargin, and this factor is largely responsible for the wound-healing activity of dalargin.

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