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QUANTITATIVE CHANGES IN CELL COMPOSITION AND VASCULARIZATION

OF ASEPTIC SKIN WOUNDS HEALING IN RATS WITHOUT TREATMENT

AND WITH STIMULATION OF REPAIR BY EXOGENOUS COLLAGEN

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Histochemical and electron-microscopic investigations on experimental and clinical material have demonstrated the beneficial effect of collagen preparations on healing of wounds of varied etiology [2, 3]. However, no quantitative morphological investigations, marked by a shift from the descriptive level to the level of objective mathematical analysis of the phenomena studied [1, 7], have hitherto been undertaken.

The aim of this investigation was to compare the dynamics of the quantitative changes in the cell composition and vascularization of aseptic skin wounds healing in rats without treatment and with stimulation of repair processes by collagen powder.

EXPERIMENTAL METHOD

Experiments were carried out on 40 male Wistar rats. A teflon ring, covered above with perforated cellophane, was inserted into full-thickness skin wounds on the animals' back, with an area of 3 cm^2, in order to exclude any effect of contraction and drying of the wound. The wounds in control animals healed without the use of any preparations. The wound surface of the rats of group 2 was treated with 20 mg of collagen powder, obtained from a solution of collagen dissolved in alkali. The animals were anesthetized with ether and killed 3, 5, 7, and 10 days after the operation (five animals at each time in each group). Histological sections were stained with hematoxylin and eosin, by Van Gieson's method and with toluidine blue, by Brachet's method, and the PAS reaction. A Stefanov's ocular grid containing 10 squares, with a side of 1 mm, was used for the investigations. In 30 randomly chosen fields of vision of the microscope, and under a magnification of 600, the neutrophilic leukocytes (NL), macrophages (Mp), fibroblasts (Fb), endotheliocytes (En), and other tissue and vascular cells present on the grid were counted. Vessels with no vertical orientation (V_{nv}), vertical vessels (V_v), and the total number of vessels (V_t) were counted. Mean values and relative percentages of cells and vessels were calculated. The results thus obtained were subjected to statistical analysis by Student's test. The results of the quantitative investigation of the cell composition and vascularization of the granulation tissue (GT), calculated per standard unit of area of the measuring grid, are given in Table 1.

EXPERIMENTAL RESULTS

In the animals of group 1 in the stage of inflammation on the 3rd day the principal cells in both absolute and relative terms were NL, and the predominant blood vessels were V_{nv}. Between the 3rd and 5th days the most rapid increase in the numbers of Mp, En, and Fb took place. For instance, the absolute number of Fb by the 5th day compared with the 3rd

TABLE 1. Absolute and Relative Numbers of Cells and Blood Vessels of GT in a Field of Vision of the Microscope of Standard Area

Type of cells and vessels		Time after wounding, days							
		3		5		7		10	
		group 1	group 2	group 1	group 2	group 1	group 2	group 1	group 2
NL	Absolute	43,22±1,56	14,08±0,50	16,62±0,40	9,73±0,24	11,96±0,27	7,63±0,30	7,56±0,30	6,63±0,25
	%	60,26	18,20	19,11	11,91	16,72	9,86	12,33	9,95
	p	—	<0,001	—	<0,001	—	<0,001	—	<0,02
Mp	Absolute	13,41±0,48	21,88±0,64	23,21±0,32	12,99±0,24	10,15±0,27	7,40±0,30	6,46±0,50	9,87±0,40
	%	18,69	28,27	26,69	15,05	14,20	9,56	10,54	14,81
	p	—	<0,001	—	<0,001	—	<0,001	—	<0,001
Fb	Absolute	13,09±0,36	36,21±0,76	42,71±0,44	53,83±0,30	44,00±0,45	54,87±0,95	42,03±0,78	45,30±0,65
	%	18,25	46,79	49,12	65,95	61,55	70,91	68,36	68,01
	p	—	<0,001	—	<0,001	—	<0,001	—	<0,002
En	Absolute	1,21±0,28	3,83±0,53	3,78±0,42	5,08±0,30	4,60±0,48	6,66±0,80	4,42±0,85	4,20±0,33
	%	1,69	4,95	4,35	6,22	6,44	8,61	7,21	6,30
	p	—	<0,001	—	<0,02	—	<0,05	—	>0,05
V_{nv}	Absolute	0,59±0,16	1,53±0,35	0,90±0,16	0,94±0,12	0,30±0,04	0,37±0,20	0,37±0,25	0,13±0,05
	%	90,77	75,37	56,60	47,47	17,05	17,62	25,17	13
	p	—	<0,02	—	>0,05	—	>0,05	—	>0,05
V_v	Absolute	0,06±0,04	0,50±0,10	0,69±0,12	1,04±0,12	1,46±0,12	1,73±0,30	1,10±0,25	0,87±0,15
	%	9,23	24,63	43,40	52,53	82,95	82,38	74,83	87
	p	—	<0,001	—	<0,05	—	>0,05	—	>0,05
V_t	Absolute	0,65±0,16	2,03±0,35	1,59±0,20	1,98±0,12	1,76±0,08	2,10±0,20	1,47±0,30	1,00±0,10
	%	100	100	100	100	100	100	100	100
	p	—	<0,001	—	>0,05	—	>0,05	—	>0,05

Legend. p relative to animals of group 1.

day was increased by 3.26 times and the number of En by 3.12 times. The increase in the number of Mp was more moderate (by 1.76 times) and with this it reached its peak and its greatest relative contribution. At the same period the absolute number of V_t was increased by 2.44 times and of V_v by 11.5 times. By the 7th day the number of NL and Mp was reduced whereas the numbers of En, Fb, and V_t reached their maximal values, and V_v reached its highest absolute and relative values. In the stage of fibrous transformation of GT the beginning of scar formation (10th day) there was some decrease in the absolute number (although their relative numbers continued to rise) of En and Fb; the numbers of V_v and V_t also decreased.

In the animals of group 2, the absolute and, in particular, the relative numbers of cells in the wounds on the 3rd and 5th days under the influence of collagen powder were almost identical with those on the 5th and 7th days in the animals of group 1. By the 3rd day there was a sharp decrease (by 3.06 times) in the absolute number of NL, whereas the number and relative proportion of Mp reached their peak values. There was a particularly sharp rise on the 3rd day in the absolute number of En (by 3.16 times) and Fb (by 2.76 times). The greatest relative number of En and Fb compared with the control was observed on the 7th day. The number of vascular elements was increased, especially in the period of FT formation. For instance, the absolute number of V_t by the 3rd day was increased by 3.12 times and the number of V_v by 8.33 times. The largest number of V_v, just as in the control, was found on the 7th day, but this value was very close to those on the 3rd and 5th days. By the 10th day the absolute number of V_{nv}, V_v, and V_t, and also the number of En, were reduced compared with those in animals of group 1.

Thus during healing of aseptic wounds in the rats of group 1 at the stage of inflammation (3rd day) the predominant cells were NL, ridding the wound of microorganisms, foreign bodies, and tissue breakdown products. The sharp increase in the numbers of En, V_t, and V_v between the 3rd and 5th days is evidence that it is during this period that vascular elements are formed most rapidly in aseptic wounds.

Maximal vascularization of GT was observed on the 7th day, for by this time the number of En and the absolute values of V_v characteristic of GT are achieved.

The most active proliferation of En coincides in time with the greatest increase in the number of Mp (cells playing a key role in the realization of functions of specific and nonspecific immunity, inflammation, and repair processes [2, 6, 8, 11, 14], in wounds. During the period of GT formation they not only carry out phagocytosis of tissue debris (destroyed NL, fibrin, etc.), but they also secrete various biologically active substances controlling intercellular and cell-tissue interactions. Considering information to show that Mp can secrete inducers of angiogenesis, stimulating En proliferation [13], it can be tentatively suggested that on the 3rd–5th day after trauma, Mp migrating into the zone of inflammation to replace NL, secrete monokines, which stimulate En proliferation and growth of vessels. The Mp also secrete fibronectin, which potentiates chemotaxis of Fb, and also monokines, which stimulate Fb proliferation [12], thus explaining one cause of the most rapid increase in the number of Fb between the 3rd and 5th days.

The small decrease in the absolute number of En and Fb by the 10th day, although their relative number continued to rise, can be explained by fibrous transformation of GT, during which some of these cells underwent destruction, but by a lesser degree than the other cells.

In the animals of group 2, under the influence of exogenous collagen considerable activation of vascularization and of GT formation was observed, especially in the early period of wound healing — on the 3rd day after the operation. The acceleration of vascularization of GT can be explained as follows. First, exogenous collagen, as a direct inducer of angiogenesis, like the collagen of the ground substance, evidently promotes adhesion, migration, proliferation, and differentiation of En. Second, in the role of an indirect inducer of angiogenesis, collagen can stimulate growth of vessels through its interaction with Mp. Collagen and its breakdown products, being chemotaxic factors for both Fb and Mp [10], increase the number of these cells in the wound [2]. By resorbing exogeneous collagen and becoming activated, Mp evidently secrete monokines, which stimulate proliferation of En and Fb and synthesis by the latter of collagen and noncollagen proteins [15], which promotes earlier vascularization and GT formation and maturation in aseptic wounds.

Active growth of blood vessels is accompanied by a marked increase in the number of Fb and the quantity of GT. The possibility cannot be ruled out, as autoradiographic studies [4] have shown, that one way whereby the number of Fb is increased is through their generation from the outer layers of the walls of small blood vessels. It is evident that not only Mp and lymphocytes, but also En participate in stimulation and regulation of growth of Fb, which is confirmed by the ability of En to secrete growth factors that stimulate Fb proliferation [9].

The decrease in the absolute number of En and, in particular, of Fb and also in the number of vessels (V_{nv}, V_v, and V_t) toward the 10th day is connected with the earlier onset of a more active stage of fibrous transformation of GT and scar formation. The increase in the number of Mp during this same period can evidently be explained by the participation of these cells in regulation of functional activity of the fibroblasts [2], which carry out phagocytosis of collagen fibrils.

The absolute and relative number of cells and vessels in healing aseptic skin wounds thus change in accordance with the stage of wound healing. In wounds healing without treatment, the most rapid increase in the number of Mp, En, and Fb is observed by the 5th day, and maximal vascularization of GT by the 7th day. In response to stimulation of repair processes by collagen the macrophagal response, proliferation of En and Fb, and vascularization of the wound are activated earlier, although the stereotyped time course of the intercellular relationships in the course of wound healing is unchanged.

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