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MICROCIRCULATION AND ANGIOGENESIS DURING WOUND HEALING BY FIRST AND SECOND INTENTION

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UDC 617-001.4-003.9-07:[616.16-005+616.16-003.9]-031.84-07

KEY WORDS: microvessels, wound inflammation, microcirculation, angiogenesis.

A favorable course of wound healing largely depends on the state of the microcirculatory bed, the depth of spread, intensity, and duration of the inflammatory reaction, and also the promptness and intensity of blood vessel formation in the wound region [2, 6, 8, 9, 11-14]. Impairment of tissue nutrition through direct injury to microvessels as a result of trauma, and the disturbance of their function during inflammation lead to the appearance of areas of primary and secondary necrosis [5, 6, 12]. Newly formed blood vessels account for the main mass of granulation tissue [2], and the accumulation of fibroblasts in the wound and collagen production, which processes are possible only if the blood supply is adequate [6, 11, 13] and, finally, an adequate blood flow in the course of healing, all exert a favorable influence on the mechanical strength of the resulting scar [9]. Changes in the microcirculation during wound inflammation [6, 8, 11, 12, 14] and the times, sequence, and possible changes in stimulation of angiogenesis [4, 11, 12] have now been well studied. However, virtually no research has been undertaken with the aim of a direct analysis and quantitative characterization of these processes under conditions close to those found during the natural course of reparative regeneration of soft tissue wounds.

The most labile parameters of the microcirculatory bed are the diameter of the microvessels and the number of capillaries simultaneously involved in the blood flow (the functioning capillary density — FCD) [7, 14]. The study of their dynamics and a description of the changes in the microcirculation during healing of experimental wounds of the subcutaneous areolar tissue were the aims of the investigation described below.

EXPERIMENTAL METHOD

Experiments were carried out on 72 Wistar rats weighing 150-200 g, divided into three groups. Under sterile conditions a linear wound of the skin and subcutaneous areolar tissue 3 cm long, in the interscapular region, was inflicted with a sharp scalpel on the 32 animals of group 1 under sterile conditions. The incision in the subcutaneous areolar tissue was closed by interrupted gauge 5-0 silk sutures on an atraumatic needle, and the skin wound was closed by a continuous intradermal silk suture. In the 32 animals of group 2 the wound was left open and infected with a suspension of a 24-h culture of *Staphylococcus aureus* (10^9 microbial cells/ml) and covered with a gauze dressing. The eight animals of group 3 were used to study the microvessels in the intact subcutaneous areolar tissue.

The microvessels were selectively stained by a modified method [10] based on the study of the spread of a peroxidase preparation of plant origin, injected into the blood stream. Workers who have used a similar method to reveal microvessels in the omentum and skeletal muscles also have noted its advantages over traditional methods, namely the possibility of detecting functioning microvessels, and the minimal deformation of the vessels compared with intravital conditions [3], so that the evaluation can be based on the criteria of biomicroscopy.

Research Institute of Laser Surgery, Ministry of Health of the USSR, Moscow. Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 105, No. 2, pp. 243-246, February, 1988. Original article submitted January 5, 1987.

TABLE 1. Functioning Capillary Density in Course of Wound Healing (in % of total volume of tissue; $p < 0.01$)

Time of observation, days	Distance from wound margin, mm (M ± m)					
	0	1	2	4	6	8
			Group 1			
1	—	Single	2,9±0,6	3,8±0,5	3,9±0,5	5,0±0,5
3	—	2,2±0,5	4,1±0,6	4,5±0,5	5,8±0,5	5,4±0,6
5	—	2,6±0,4	3,6±0,4	4,5±0,4	5,7±0,4	6,0±0,4
7	Single	2,9±0,4	3,4±0,5	4,9±0,4	5,4±0,5	5,7±0,5
10	2,8±0,6	5,1±0,6	5,0±0,5	5,3±0,6	5,9±0,6	6,0±0,6
15	5,5±0,5	5,8±0,5	5,8±0,5	5,8±0,5	5,8±0,5	5,8±0,5
20	4,6±0,4	5,7±0,5	5,7±0,5	5,7±0,5	5,7±0,5	5,7±0,5
			Group 2			
1	—	—	—	2,2±0,4	4,6±0,5	6,0±0,6
3	—	—	1,9±0,5	5,5±0,4	5,9±0,4	5,7±0,6
5	—	1,2±0,4	2,5±0,4	5,4±0,6	5,2±0,5	5,6±0,5
7	—	1,9±0,5	3,6±0,6	5,5±0,5	5,9±0,6	5,4±0,5
10	Single	2,2±0,5	5,3±0,5	5,3±0,6	5,7±0,6	5,6±0,5
15	2,1±0,5	5,8±0,5	6,1±0,5	6,3±0,6	5,7±0,5	5,5±0,6
20	4,7±0,6	5,4±0,5	5,4±0,5	5,4±0,5	5,4±0,5	5,4±0,5

A solution of peroxidase in a dose of 20 mg of the pure preparation per 100 g body weight was injected into the blood stream and, 5 min later, the wound tissues were fixed intravitally with 2.5% glutaraldehyde solution; 30 min later the wound was excised and film preparations were made, washed in phosphate buffer (pH 7.2) to remove the fixative, incubated with DAB substrate in the presence of hydrogen peroxide, dehydrated, cleared, and mounted in balsam. Material was studied 1, 3, 5, 7, 10, 15, and 20 days after wounding.

Intensely stained functioning and pale (stained on account of erythrocytic peroxidase) nonfunctioning microvessels, regions of spread of the enzyme outside the microvessels, evidence of their increased permeability, and also macrophages, polymorphonuclear leukocytes, and mast cells could be clearly differentiated in the preparations. FCD were counted by means of an Avtandilov grid [1] at distances of 0, 1, 2, 4, 6, and 8 mm from the edge of the wound, and the diameters of the arterioles and venules were measured with an ocular micrometer at a depth of 4-5 mm.

EXPERIMENTAL RESULTS

The microcirculatory bed of the intact subcutaneous areolar tissue has a netlike structure; the mean diameter of the arterioles was 33 ± 1.5 μ and of the venules 49 ± 1.9 μ. Capillaries from 3 to 10 μ in diameter formed dense plexuses with numerous anastomoses. FCD accounted for 5.5 ± 0.3% of the total volume of the tissue.

A zone of destructive changes in the microvessels at a depth of 0.8-0.3 mm was observed in the wounds of the animals of group 1 24 h after trauma; in it were observed marked deformation and fragmentation of the arterioles and venules, numerous hemorrhages, abundant neutrophilic infiltration, and diffuse staining of the tissues with the substrate. A similar pattern also was found in group 2 but in this case the zone of destruction was 2.6 ± 0.6 mm deep.

Beyond the zone of destruction lay a zone of marked microcirculatory disturbances, characterized by hemoconcentration, aggregation of erythrocytes, widespread stasis, and deformation and blurring of the outlines of the afferent and efferent vessels. The infrequent functioning capillaries were mainly short shunts connecting arterioles and venules. The depth of this zone was 1.6 ± 0.3 mm in group 1 and 3.4 ± 0.5 mm in group 2.

The beginning of the next zone, one of moderatly severe microcirculatory disturbances, was defined by predominance of vessels stained with exogenous peroxidase in the visual field. Capillaries were tortuous, dilated, and congested and contained aggregates of erythrocytes resembling "rouleaux"; the venules also were tortuous, with foci of perivascular diapedesis and pavementing of leukocytes. In both groups 1 and 2 the external boundary of this zone lay outside the limits of the preparations, i.e., more than 8 mm; with increasing distance

from the wound edge the features of the inflammatory reactions were less frequently found, and this was accompanied by restoration of the normal FCD (the results of morphometry are given in Table 1).

On the 3rd day of observation contraction of the zone of destruction in group 1 to 0.3 ± 0.1 mm and in group 2 to 1.3 ± 0.3 mm and of the zone of marked circulatory disturbances to 1.8 ± 0.2 and 1.6 ± 0.5 mm, respectively, was observed. Among cells infiltrating the inflammatory focus mononuclear phagocytes were seen, and on the boundary between the zone of marked and moderate disturbances there were islets of newly formed capillaries, running perpendicularly to the wound edge.

By the 5th day the site of the zone of destruction was occupied by a narrow band of tissue, free from blood vessels. Its width was 0.4 ± 0.1 mm in group 1 and 0.8 ± 0.2 mm in group 2. The zone of maked disturbances was still present only in group 2, in which it measured 1.5 ± 0.4 mm. The predominant cell population near the wound edge in group 1 was the macrophages, but in group 2 mixed infiltration was still present. In both groups 1 and 2, abundant growth of blood vessels, arising from the kinks or aneurysmal dilatations of the capillaries, small venules and, less frequently, arterioles were observed and could be traced to a depth of 10-12 mm. However, the plasma was stained by exogenous peroxidase in by no means all of the newly formed capillaries although they already contained erythrocytes.

On the 7th day the wound canal in group 1 was covered by capillary anastomoses connecting the opposite edges of the wound. In group 2, the predominant mononuclears, as before there were infrequent polymorphonuclear leukocytes, and in a large proportion of the newly formed capillaries inflammatory changes were present to a varied degree, causing their partial destruction. The zone of marked disturbances measured 1.4 ± 0.4 mm. At a depth down to 5-7 mm circular foci of infiltration containing neutrophils and macrophages could be seen, surrounded by an area of rather more numerous newly formed capillaries.

By the 10th day arterioles and venules were differentiated among the growing vessels. In group 1 a well-developed capillary network could be identified in the center of the wound. In group 2 individual functioning capillaries appeared to be actually at the wound edge, and the zone of marked disturbances was narrowed to 0.7 ± 0.2 mm.

Toward the 15th day an epithelized scar formed in all the animals of group 1. In group 2 the wound was completely healed in one of the nine remaining rats. The zone of marked disturbances was absent in preparations obtained from the rats of this group. Complete restoration of the number of functioning capillaries was observed now at a distance of 1 mm from the wound edge.

On the 20th day a ramified capillary network with evidence of moderately severe inflammation was located in group 2 beneath the scar.

More marked dilatation of the capacitive vessels in the wounds of the rats of group 2 (up to 80 μ) was found by morphometry of the arterioles and venules during healing on the 1st to the 7th days after trauma, whereas the diameter of the arterioles showed no significant fluctuations at these times. Dilatation of the venules to 60 μ was observed in group 1 on the 1st-3rd days after trauma. Dilatation of the capacitive vessels in this case was evidence of the intensity of the inflammatory reaction [7, 14], and thus confirmed the results of the direct observations.

The deeper injury to the tissues on account of infection and additional mechanical trauma, and also the presence of an obstacle to the early anastomosis of the newly formed vessels in the opposite walls of the wound, observed during healing of wounds by second intention, thus contribute to a more intensive and prolonged inflammatory reaction. Meanwhile, the marked similarity between changes in the microcirculation and the times of blood vessel formation in groups 1 and 2 confirms the concept of the quantitative character of differences during wound healing by first and second intention [6, 8, 12].

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