ROLE OF THE MICROBIAL FACTOR, NECROTIC MASSES, AND FOREIGN BODIES IN THE DEVELOPMENT OF WOUND SUPPURATION

E. M. Danilina, S. A. Pisarzhevskii, G. N. Dudnikova, and A. A. Karelin

UDC 617-001.4.002.3]-02:[617-001.46+617-001.4-022.7+617-001.4

KEY WORDS: wound suppuration; necrosis; wound microflora; nucleic acids; proteolytic enzymes.

For suppuration to develop in a wound the total number of microbial cells per gram of wound tissue must exceed a certain critical level which depends on the general state of the host and the state of the wound tissues [7]. The influence of the various factors on the development and course of suppuration has been studied mainly in clinical material [1], which is difficult to analyze because of its heterogeneity. Models of suppurating wounds in laboratory animals such as rats could not be found in the literature.

The aim of the present investigation was to create an easily reproducible model of wound suppuration in rats and to study the effects of the microbial factor, necrotic masses, and foreign bodies on the development of wound infection and on some parameters of its course.

EXPERIMENTAL METHOD

Experiments were carried out on 338 noninbred albino rats weighing 250-300 g. In the experiments of series I, under ether anesthesia, a full-thickness excised wound of the skin was inflicted in the region of the spine, measuring 2.0 × 2.5 cm. The rats were then divided into two groups: the animals of group 1 had a foreign body (a Teflon ring, described by Slutskii [5]) sutured into the region of the wound, whereas in the animals of group 2 no foreign body was present in the wound. Each group of rats was then divided into three subgroups (A, B, and C). In the animals of subgroup A, the muscles in the floor of the wound were made necrotic by injection of 1 ml of an 8% solution of $CaCl_2$ immediately after the operation; in the rats of subgroup C the muscles of the floor of the wound were made necrotic by application of a cotton swab soaked in 25% NaOH solution for 1 min immediately after the operation; in subgroup C the muscles were not made necrotic. Immediately after the operation a culture of the pathogenic Baikov strain of *Staphylococcus aureus* was introduced into the wound in doses of 10^5, 10^6, 10^7, 10^8, and 10^9 microbial cells in 1 ml physiological saline per animal. Rats not receiving the microorganisms served as the control. As the additional control, in some rats of subgroups 2A and 2B, from the 1st through the 9th day after the operation the wounds were treated with a 3% solution of boric acid; another batch of animals from the same subgroups received injections of the sodium salt of oxacillin into the wound in a dose of 20 mg in 1 ml physiological saline per rat, also from the 1st through the 9th day after the operation.

In the experiments of series II, in order to discover the role of different microorganisms in the development of wound suppuration, the rats of subgroup 2A were divided into three groups (2A1, 2A2, and 2A3): the nonpathogenic strain 209P of *Staph. aureus* was introduced in a dose of 10^9 microbial cells in 1 ml physiological saline per animal into the necrotized muscles in the floor of the wound of rats of group 1A1 on the 3rd and 5th days after the operation, rats of group 2A2 received a culture of *Escherichia coli* 10K, and the rats of group 2A3 a culture of *Staph. aureus* strain Baikov at the same time and in the same doses. Rats whose wounds were treated with oxacillin, just as in the experiments of series I, served as the control.

To assess the course of wound healing, in the experiments of series I daily observations were made of the state of the wound from the 1st through the 17th days after the operation, paying particular attention to the appearance of the suppurative discharge. In the rats of subgroup 2A, wound biopsy was performed under sterile conditions on the 5th, 9th, 12th, and

Laboratory of Biochemistry and Laboratory of Polymers in Medicine, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 3, pp. 31-35, March, 1983. Original article submitted July 19, 1982.

0007-4888/83/9503-0289$07.50

TABLE 1. Clinical Manifestation of Wound Infection in Rats 15 Days after Operation, Depending on Various Factors

Foreign body	Necrotizing agent	Uninfected wound	Dose of Staph. aureus, strain Baikov, microbial cells				
			10^5	10^6	10^7	10^8	10^9
Teflon ring	1 ml of 8% $CaCl_2$ solution	+	+	+	+	+	+
	25% NaOH solution	+	+	+	+	+	+
	Without necrotization	—	—	—	—	—	—
No foreign body	1 ml of 8% $CaCl_2$ solution	+	+	+	+	+	+
	25% NaOH solution	+	+	+	+	+	+
	Without necrotization	—	—	—	—	—	—

Legend. +) Obvious clinical picture of wound infection, —) absence of such a picture, clinically clean wound.

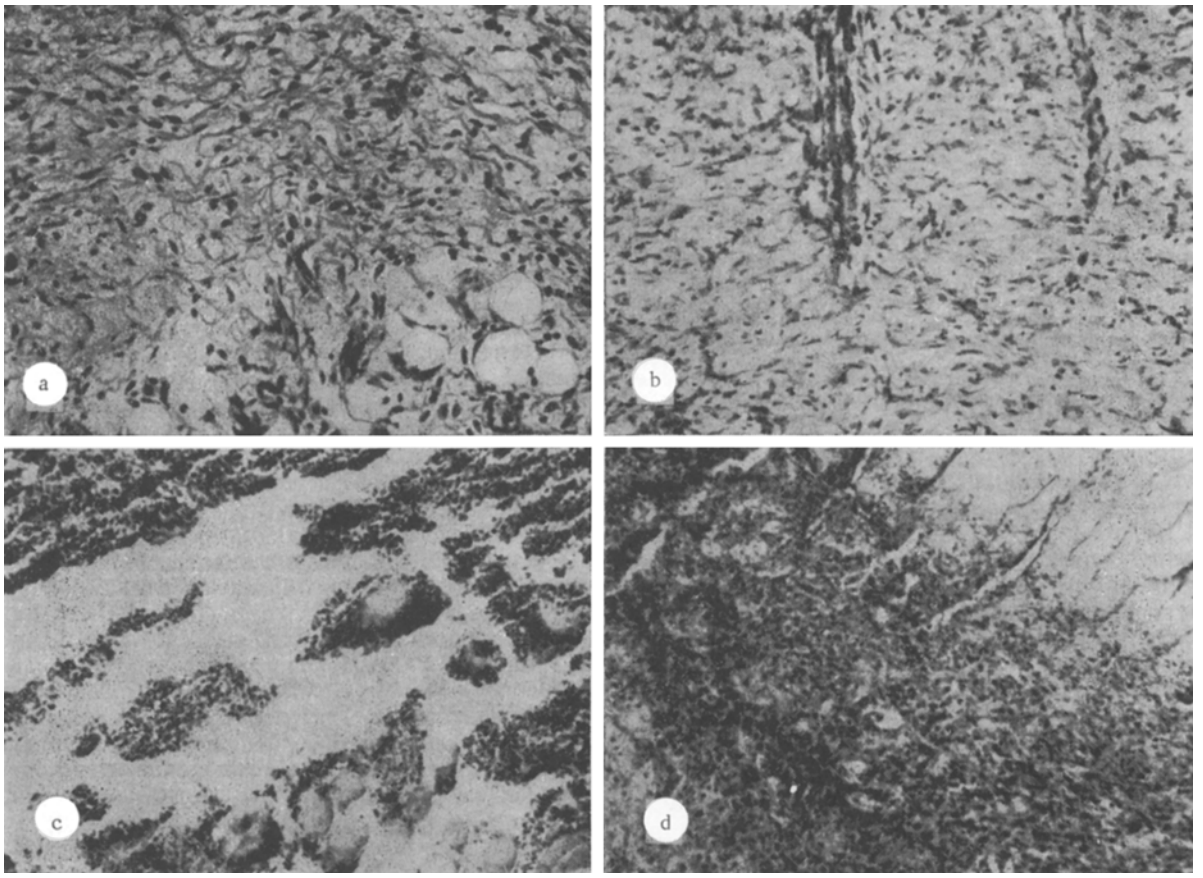

Fig. 1. Wounds on 9th day after operation: a) flora of clean wound lined with maturing granulation tissue, b) newly formed capillaries penetrate into surface layers of clean wound, c) zone of edema and leukocytic infiltration in suppurative wound, d) leukocytic infiltration and fibrinous masses on surface of suppurative wound. Hematoxylin, 160×.

17th days by the method in [8], and homogenates of the specimens were plated on Petri dishes with nutrient agar, and incubated for 24 h at 37°C. Films were prepared from the cultures of microorganisms grown in this way, stained by Gram's method, and examined under the microscope

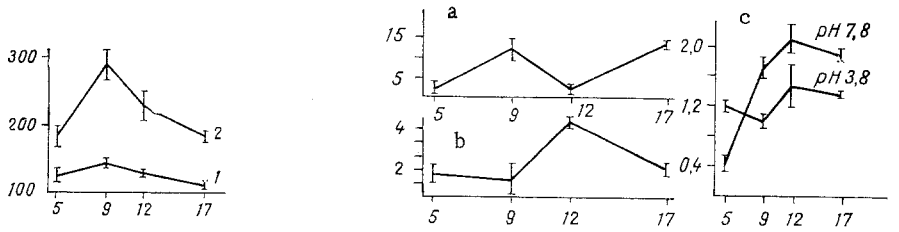

Fig. 2. Time course of BII level in blood of rats with clean (1) and suppurating (2) wounds. Abscissa, time after operation (in days); ordinate, BII level (in % of its level in intact animals).

Fig. 3. Time course of DNA content (a), RNA/DNA ratio (b), and total proteolytic activity (c) in suppurating wound tissues of rats. Abscissa, time after operation (in days); ordinate: a) DNA concentration (in μg/mg dry weight of tissue), b) RNA/DNA ratio, c) total proteolytic activity (in μg tyrosine/min/mg wet weight of tissue).

under a magnification of 90 × 15. A histological study of the wounds was carried out at the same times and the total number of microbial cells in 1 g of wound tissue [3], the content of nucleic acids [2], the total proteolytic activity at pH 3.8 and 7.8 [4] (using human serum albumin from Reanal, Hungary, as the substrate), and also the level of potential biochemical indicators of infection (BII) in an $HClO_4$-supernatant of heparinized blood, measured at 388 nm by the method in [9], were determined. The same parameters were studied at the same times in animals with clean excised wounds. In the experiments of series II, the total nucleic acid content and the total proteolytic activity in the wound tissues at pH 7.8 were determined on the 7th and 11th days after the operation, films prepared from wound biopsy material were examined under the microscope, and histological investigation of the wounds was carried out just as in series I.

EXPERIMENTAL RESULTS

According to the results of the investigations the presence of necrotic masses is a necessary and sufficient condition for clinical manifestation of infection in wounds in rats. The result was independent of the infecting dose of microorganisms, the presence of a foreign body, and the nature of the necrotizing agent, and infection was not prevented by the use of local antibacterial preparations (Table 1).

The Gram-positive coccal wound microflora with necrosis and obvious clinical manifestations of infection, which predominated in the early stages, gave way to Gram-negative by the 12th day, whereas in wounds without necrosis a Gram-positive coccal microflora predominated at all times. A similar change in the microflora in the wound is often observed in clinical practice [3]. In suppurative wounds treated with oxacillin the Gram-negative microflora predominated until the 9th day, and after treatment with oxacillin ended the Gram-positive coccal microflora was restored. In rats of subgroup 2A2, despite wound infection in the early stages after the operation with high doses of *E. coli*, the predominant microflora in the wounds until the 12th-14th day was Gram-positive, coccal in type. We know that *E. coli* and *Bacillus pyocyaneus*, which are often found in suppurative wounds, are constant normal inhabitants of the intestine and are found in partially decomposed food [3]. Possibly replacement of the Gram-positive wound microflora by Gram-negative is associated with the fact that during decomposition of the necrotic masses the Gram-negative microflora becomes more competitive and it gradually displaces the Gram-positive flora, which is more adapted to existence in the early stages of decomposition of the necrotic masses.

For further investigations a model was chosen without introduction of the microorganism (subgroup 2A) as easily reproducible and, because of self-induced development of clinical manifestation of infection in the wound, the most adequate model of the corresponding pathology in man. In models of wounds in rats of this group, a large quantity of yellowish green pus could be seen visually from the 5th-7th through the 14th-15th day. Suppuration gradually subsided, and by the 17th-20th day complete rejection of the suppurative necrotic masses took place, and the wounds healed by the 35th day.

On the 5th day after the operation the suppurating wounds consisted of a cavity filled with suppurative-necrotic masses, the floor and edges of which were formed by necrotic muscles. Around the muscle fibers massive leukocytic infiltration and area of edema and hemorrhages could be observed. Growth of islets of granulations was observed on the 9th day (Fig.

1). Massive proliferation of granulation tissue occurred on the 12th day. In the substance of the granulation tissue microabscesses and hemorrhagic foci were present. Nonepithelized areas of the wounds were covered by a thick leukocytic-necrotic barrier. The anterior border of the regenerating epithelium, growing above the granulations, in places underwent destruction by leukocytes. Beneath the epithelium fibrous masses and hemorrhages were observed. The process of cleansing of the wound surface was not complete until the 17th day. By that time the wounds were filled with granulation tissue. Signs of maturation could be distinguished in its lower layers, but in the upper layers small hemorrhagic foci and leukocytic infiltration still remained. Active epithelization of the wound edges was in progress. By the 17th day the number of microbial bodies per gram of tissue was 10^4, compared with 10^6 on the 5th-12th days. Unlike suppurating wounds in the control, by the 5th day after the operation the wounds were filled with granulation tissue. The islets of granulation had a tendency to merge. The wound surface was covered by a thin leukocytic-necrotic barrier. Inflammatory changes in the wounds were moderately expressed. By the 9th day the control wounds were completely filled with granulation tissue, in the lower layers of which evidence of maturation could be seen (Fig. 1). At the wound edges activation of the epithelium was noted. Epithelization of the control wounds was complete by the 23rd day. In the process of healing of the wounds on the control rats, the largest number of microbial bodies per gram of tissue was 10^4.

Determination of BII in the blood of rats with suppurating wounds showed elevation of their level compared with the control, but on the 9th day that level reached a maximum of 102% ($P < 0.01$; Fig. 2). Determination of BII is evidently a promising procedure for diagnosis and prognosis of the course of wound healing.

There are indications in the literature of a high DNA concentration in purulent discharges from wounds [6]. The quantity of purulent exudate in suppurating wounds on the 9th day reached its highest level and this was accompanied by a sharp increase in the DNA concentration in the wound (by 5.83 times; $P < 0.02$) compared with that on the 5th day (Fig. 3a). On the 12th day the DNA level was 6.1 times lower ($P < 0.02$) than on the 9th day, which correlated with a decrease in the quantity of purulent exudate, but it rose again so that on the 17th day it was 6.71 times higher ($P < 0.05$) than on the 12th day, probably due to intensification of proliferation. The rise in the RNA/DNA ratio on the 12th day by 3.75 times ($P < 0.01$) compared with its value on the 5th day (Fig. 3b) was evidently associated with the beginning of repair processes, and its fall on the 17th day by 64% compared with its level on the 12th day was associated with inhibition of the biosynthetic activity of the cells in the course of proliferation.

Proteolytic activity in the wound increased until the 12th day, then remained at approximately the same level (Fig. 3c); the total proteolytic activity at pH 7.8, moreover, was higher by 49.4% ($P < 0.05$) than at pH 3.8 on the 12th day and by 42% ($P < 0.05$) on the 17th day. Evidently in the course of suppuration and sloughing of dead tissues proteolysis is intensified on account of an increase in activity of neutral proteases.

Infection of wounds with necrosis by a strain of pathogenic staphylococcus causes lowering of the RNA/DNA ratio in the wound tissues on the 11th day by 2.38 times ($P < 0.05$), an increase in total proteolytic activity at pH 7.8 by 25.0% ($P < 0.05$), and an increase in DNA accumulation by 68.0% ($P < 0.05$) compared with the corresponding parameters in wounds treated with oxacillin. Introduction of a nonpathogenic staphylococcus into the wound also increased the DNA content in its tissues by 19.1% ($P < 0.05$) compared with that in wounds treated with the antibiotic. No significant differences could be found on histological investigation of the wounds in these animals. Infection of the wound with staphylococci probably accelerates disintegration of necrotic tissues but has no significant effect on the intensity of repair processes.

LITERATURE CITED

1. D. Alexander and R. Good, Immunology for Surgeons [Russian translation], Moscow (1974).
2. E. M. Zhantieva and N. V. Bogoyavlenskaya, Vopr. Med. Khim., No. 5, 545 (1972).
3. Wounds and Wound Infection [in Russian], Moscow (1981).
4. A. P. Rukhlyadeva and G. V. Polygalina, Methods of Determination of Hydrolytic Enzymes [in Russian], Moscow (1981).
5. L. I. Slutskii, Biochemistry of Normal and Pathologically Changed Connective Tissue [in Russian], Leningrad (1969).
6. V. I. Struchkov et al., The Suppurating Wound [in Russian], Moscow (1975).
7. S. D. Elek, Ann. N. Y. Acad. Sci., 65, 65 (1956).
8. T. Krizek and M. Robson, Am. J. Surg., 130, 579 (1975).
9. M. C. Powanda, J. Dubois, et al., J. Lab. Clin. Med., 97, 679 (1981).