TABLE 1. Formation (in vol. %) of Collagen-like Material in Left and Right Ventricular Myocardium of Rabbits after Injection of Diphtheria Toxin Alone and Preceded by Injection of Large Doses of Retinol and α-Tocopherol

Experimental conditions	Time of investigation			
	background	24 h	72 h	6 days
Diphtheria intoxication				
Left ventricle	3,8±0,36	5,77±0,45	3,27±0,34	6,03±0,45
Right ventricle	6,95±0,42	13,77±0,69	5,67±0,44	5,73±0,45
Diphtheria intoxication preceded by injection of vitamins A and E:				
Left ventricle	6,77±0,48	12,13±0,65	2,87±0,31	6,27±0,46*
Right ventricle	6,28±0,45	16,77±0,76	3,9±0,37	7,97±0,52

Legend. Asterisk indicates that mean values within the group do not differ significantly from background values.

also, consequently, its contractile properties. Moreover, these perivascular "cuffs" prevent transport of oxygen and nutrients into the myocardium. This protective reaction thus begins to acquire a pathogenetic character.

LITERATURE CITED

1. V. A. Frolov, Philosophical and Social Problems in Biology and Medicine [in Russian], Moscow (1977), pp. 75-84.
2. V. A. Frolov, G. Mall, T. Mattfeldt, et al., Dokl. Akad. Nauk SSSR, 256, No. 2, 503 (1981).
3. V. A. Frolov and G. A. Drozdova, The Hypertensive Heart [in Russian], Baku (1984).
4. P. Antoni, Karen L. di Sant Agnese, et al., Am. J. Clin. Path., 81, No. 12, 25 (1984).
5. G. Mall, T. Mattfeldt, P. Rieger, et al., Basic Res. Cardiol., 77, No. 1, 57 (1981).
6. K.-U. Thiedeman, Herzhypertrophie, Berlin (1985), pp. 48-70.

CYTOPHOTOMETRIC STUDY OF MYELOPEROXIDASE IN BLOOD NEUTROPHILS AND WOUND EXUDATE DURING EXPERIMENTAL WOUND HEALING

L. A. Mamedov, A. V. Nikolaev, V. V. Zakharov, A. B. Shekhter, and Yu. R. Khrust

UDC 617-001.4-003.9-07:616.155.34-008.931+617-001.4-008.831/:577.152.1-074

KEY WORDS: wound healing; inflammation; myeloperoxidase; neutrophils.

Myeloperoxidase (MP) is the main component of the most important bactericidal system of neutrophilic granulocytes [6, 9]. MP activity is found in virtually all cells [5]. Its content in neutrophils varies depending on the pathological state [5, 7], including in many surgical diseases [2]. However, the dynamics of the changes in MP activity during wound healing has not yet been explained, although the treatment of wounds remains an urgent problem in modern surgery [4].

This paper describes a cytophotometric investigation of the content of the end product of the cytochemical reaction for MP in the blood neutrophils and wound exudate during healing of full-thickness aseptic and infected skin wounds in rats.

I. M. Sechenov First Moscow Medical Institute. M. V. Lomonosov Moscow University.

(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 12, pp. 741-743, December, 1987. Original article submitted April 3, 1987.

0007-4888/87/0012-1752$12.50

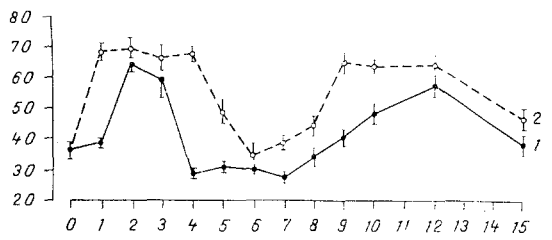

Fig. 1

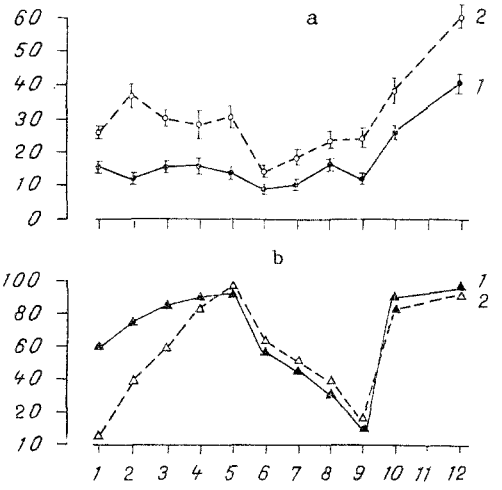

Fig. 2

Fig. 1. MP activity in blood neutrophils from rats with aseptic (1) and infected (2) wounds. Abscissa, time after operation (in days); ordinate, intensity of reaction for MP (in optical density units).

Fig. 2. MP activity in neutrophils from wound exudate of rats with aseptic (1) and infected (2) wounds. Abscissa, time after operation (in days); ordinate: a) intensity of reaction for MP (in optical density units), b) proportion of MP-positive neutrophils in exudate (in %).

EXPERIMENTAL METHOD

Two series of experiments were conducted on 220 male Wistar rats weighing 200-210 g. Full-thickness aseptic and infected skin wounds, with an area of 400 mm^2, constituted the experimental model. The model of an aseptic wound was created under general hexobarbital anesthesia (150 mg/kg, intramuscularly). The dorsal aspect of the cervical region of the rats, an area of skin with the underlying cellular tissue and superficial fascia was removed under aseptic conditions after preliminary removal of the hair cover, and plastic rings covered on the top with perforated film were inserted into the wound. To obtain a model of an infected wound, the edges and floor of the wound were additionally traumatized with toothed forceps and 0.5 ml of a suspension of a 24-h culture of a pathogenic staphylococcus ($1.5 \cdot 10^9$ microbial cells in 1 ml of physiological saline) was injected inside the ring on the wound surface. The rings were removed after 2 days. Before the operation and daily for 10 days thereafter, and also on the 12th and 15th days of the experiment, blood films were taken from the caudal vein. Squash preparations from the wound surface were obtained at the same times. Some of the films and squash preparations were stained for morphological analysis by the Romanovsky–Giemsa method, the rest by Loele's method [11] for MP activity, without counterstaining of the nuclei. The content of the end product of the cytochemical reaction for MP was determined cytophotometrically in 50 morphologically preserved neutrophils in each preparation. Measurements were made on the MIF-k cytophotometer [8], built in the Department of Cytology and Histology, Biological Faculty, M. V. Lomonosov Moscow University. This apparatus is based on the principle of the logarithmic screen and it is distinguished by high resolving power and speed of action, which make it particularly valuable for measuring the intensity of cytochemical reactions [3]. Each experimental point corresponded to eight animals and, consequently, the results for each point were based on measurement of the intensity of the reaction for MP in 400 neutrophils. The significance of differences between individual points was determined by Student's t test.

EXPERIMENTAL RESULTS

MP activity in the blood neutrophils showed similar changes in the two series (Fig. 1): an initial rise of the curve until the 4th-5th day was followed by a fall, to reach a minimum of activity on the 5th-6th day. Later a second increase of MP activity was observed until the 12th day, after which it returned to its initial level. Except on the 2nd and 6th days, differences between the series were significant at all points ($p < 0.05$). The coefficient of variation in the two series varied over the range from 3 to 25%.

Absolute values of MP activity were higher in the series of experiments with an infected wound than in the series with an aseptic wound at all stages of the experiment. On the 1st day after the operation MP activity in the blood neutrophils of rats with infected wounds was already almost twice as high as its initial level, whereas in animals with an aseptic wound a significant increase in MP activity was observed only on the 2nd day.

The initial rise of MP activity was evidently partly attributable to the arrival of the bone-marrow reserves of neutrophils, which have higher MP activity [7], in the peripheral blood stream. Meanwhile the increase in MP activity was probably due also to an increase in MP production in neutrophils in response to operative trauma and bacterial infection [5]. The second rise of MP activity was evidently connected with this second factor only.

MP activity in the neutrophils of the wound exudate also was higher in animals with a suppurative wound (Fig. 2a). This is in agreement with data obtained by other workers [5, 6] showing an increase in the peroxidase activity of neutrophils before phagocytosis. However, MP activity on the 1st day was found in only 2% of neutrophils in suppurative wound exudate (Fig. 2b: 2), and it was absent in the rest, evidently due to its utilization in phagocytosis (nearly all neutrophils of the wound exudate were in various stages of phagocytosis). Processes of phagocytosis of microorganisms were largely complete by the 4th-5th day, but in individual infected wounds bacteria were present until the 12th day. By the 5th day the percentage of MP-positive neutropils in the suppurative wound increased to 95, and reached the level of the corresponding parameter in the aseptic wound (Fig. 2b: 1). Such an increase in the proportion of MP-positive neutrophils is indirect evidence in support of completion of the process of phagocytosis and renewal of the neutrophil composition of the exudate through the arrival of fresh cells from the blood stream.

The decrease in the proportion of MP-positive neutrophils observed soon after this stage was linked with their degeneration and probably reflected a decrease in the number of fresh neutrophils arriving in the wound. During this period extracellular concentrations of MP were frequently found, especially in aseptic wounds, where they were released from neutrophils [1, 10], but were not yet being utilized in phagocytosis, for microorganisms were absent both in the aseptic wounds and in the majority of infected wounds. MP activity in individual neutrophils varied during this period within wide limits (coefficient of variation 45% at times).

On the 10th-12th day the percentage of MP-positive cells and MP activity of the cells were sharply increased in both series of experiments. This phenomenon is linked with the fact that most neutrophils found in squash preparation came from the blood as a result of trauma to the granulations by the slide, i.e., essentially they were blood and not exudate neutrophils. This hypothesis was confirmed by the presence of numerous erythrocytes in these preparations.

The results of this investigation are thus evidence that MP activity in the neutrophils changes during wound healing, reflecting the character and stages of the course of the wound process and the response of the host to it. MP activity was significantly higher in blood and wound exudate neutrophils in animals with infected wounds. This reflects the intensity and duration of the inflammatory process and may be of prognostic importance.

LITERATURE CITED

1. M. M. Zhekova and I. P. Ashmarin, Zh. Mikrobiol., No. 3, 70 (1976).
2. B. S. Kaplan, "Importance of enzyme activity of peripheral blood leukocytes in the diagnosis of acute destructive processes in the abdomen," Author's Abstract of Dissertation for the Degree of Candidate of Medical Sciences, Moscow (1972).
3. G. I. Kozinets, V. M. Kotel'nikov, and V. E. Gol'dberg, Cytophotometry of Hematopoietic Cells [in Russian], Tomsk (1976).
4. M. I. Kuzin and B. M. Kostyuchenok, Wounds and Wound Infection [in Russian], Moscow (1981).
5. V. T. Morozova, Laboratory Diagnosis of Leukemias [in Russian], Leningrad (1977).
6. V. E. Pigarevskii, Granular Leukocytes and Their Properties [in Russian], Moscow (1978).
7. F. G. J. Hayhoe and D. Quaglino, Haematological Cytochemistry, London (1980).
8. Yu. R. Khrust, L. L. Litinsyaka, S. A. Cheptsov, et al., Tsitologiya, 17, No. 8, 997 (1975).
9. M. G. Shafran, Usp. Sovrem. Biol., 92, No. 3 (6), 265 (1981).
10. D. F. Bainton, J. Cell Biol., 58, 249 (1973),
11. W. Loele, Dtsch. med. Wochenschr., 62, 2004 (1936).