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BLOOD SERUM AND GRANULATION TISSUE ASCORBIC ACID AND HYDROXYPROLINE LEVELS IN RATS WITH ASEPTIC AND INFECTED WOUNDS

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UDC 617-001.4-022.7-07:616.153:577.164.2]-092.9-07

KEY WORDS: wound, inflammation, ascorbic acid, hydroxyproline.

Determination of the hydroxyproline level, an indicator of collagen accumulation, i.e., of scar healing of a wound defect, is used as an objective criterion in the quantitative evaluation of the course of wound healing [2, 3, 6]. Hydroxyproline formation is dependent on the presence of ascorbic acid, which participates in the oxidation of proline into hydroxyproline, and also influences the biosynthesis of other proteins [1, 9, 13]. The ascorbic acid level in granulation tissue also characterizes the state of oxidation-reduction processes in the wound [5, 8, 12, 15]. It also has a role in stress situations (response to pathogenetic factors, trauma, etc.) [10]. Meanwhile the dynamics of the ascorbic acid and hydroxyproline levels during healing of infected wounds, and its comparison with that during the healing of uncomplicated wounds, has been inadequately studied.

The aim of this investigation was to determine ascorbic acid and hydroxyproline levels in the blood serum and granulation tissue of rats with aseptic and infected wounds.

EXPERIMENTAL METHOD

Experiments (two series) were carried out on 220 male Wistar rats weighing 200-210 g. Aseptic and infected full-thickness wounds with an area of 400 mm^2 served as the experimental model. The model of an aseptic wound was created by the method described previously [4]. To obtain the 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 ($1.5 \cdot 10^9$ bacterial cells in 1 ml physiological saline) was introduced into the wound surface. At intervals, on the 1st-10th, 12th, and 15th days after the operation concentrations of hydroxyproline and ascorbic acid were determined in the tissues in the region of the wound (granulation tissue), and the serum ascorbic acid level also was determined at the same times and before the operation. Hydroxyproline was estimated photometrically [14] and ascorbic acid titrometrically [11]. At each time point 8 to 10 rats were used, and killed by decapitation. The wound tissues of five animals also were studied daily histologically (staining with hematoxylin and eosin, toluidine blue, and by van Gieson's, venules, arterioles) in the course of all phases of wound healing was estimated in each animal on a 5-point system (from 0 to 4). The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Analysis of the data showed (Fig. 1a) that in both aseptic and infected wounds there was a significant increase in the ascorbic acid concentration in the granulation tissue

I. M. Sechenov First Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR, V. V. Kovanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 9, pp. 355-358, September, 1988. Original article submitted December 18, 1987.

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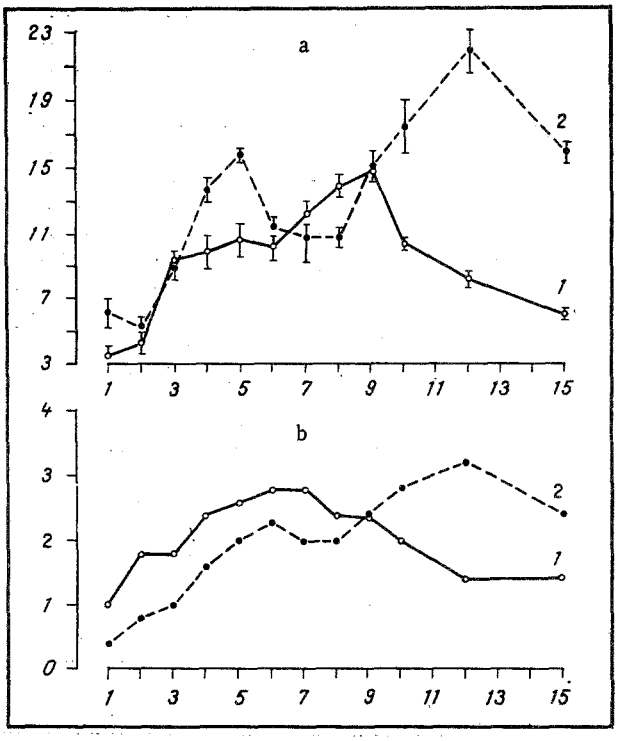

Fig. 1. Dynamics of ascorbic acid concentration (a) and number of microvessels (b) in granulation tissue of aseptic (1) and infected (2) wounds in rats. Abscissa, time after operation (in days); ordinate: a) ascrobic acid concentration (in mg%), b) number of points.

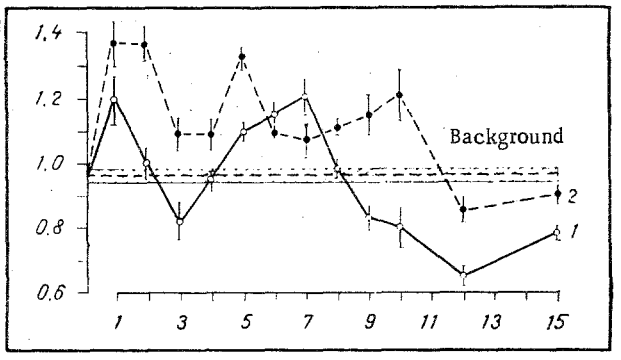

Fig. 2. Dynamics of serum ascorbic acid level (in mg%) in rats with aseptic (1) and infected (2) wounds. Remainder of legend as to Fig. 1.

starting with the 3rd day after the operation, i.e., in the phase of inflammation. The ascorbic acid concentration in the granulation tissue of aseptic wounds reached a maximum on the 8th-9th day, and thereafter gradually declined, so that by the end of the experiment it was close to the level on the first 2 days, before any granulation tissue had formed. A further increase in the ascorbic acid concentration in the granulation tissue was observed in the infected wounds at this same time (after the 9th day).

Of all the morphological parameters of granulation tissue, that whose time course most closely resembled that of the ascorbic acid concentration in it was the degree of vascularization (the number of microvessels) of the granulation tissue. As Fig. 1 shows, from the 9th day after the operation the number of microvessels in the granulation tissue of aseptic wounds decreased on account of transformation of the granulation tissue into fibrous and scar tissue. In the infected wounds the increase in the number of microvessels continued up to the 12th day, for in infected wounds maturation of granulation tissue and its conversion into scar tissue are delayed.

Reduction of vascularization during scar healing of aseptic wounds, leading to a fall in the level of oxidation-reduction processes, and also a simultaneous decrease in proliferation of fibroblasts, producing ascorbic acid [5], together lead to a fall in the level of

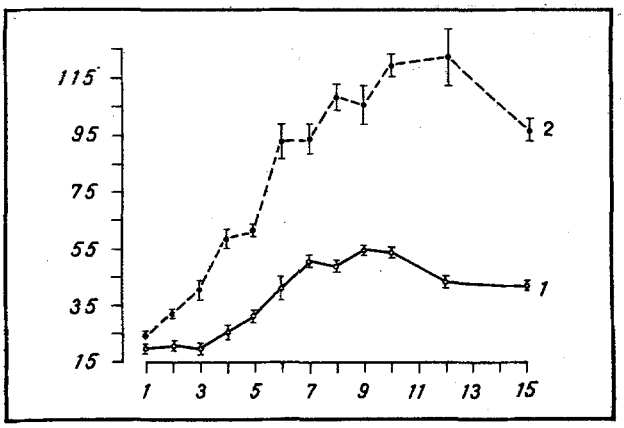

Fig. 3. Dynamics of hydroxyproline concentration (in mg%) in granulation tissue of aseptic (1) and infected (2) wounds in rats. Remainder of legend as to Fig. 1.

the latter. The ascorbic acid concentration, like the number of vessels and fibroblasts in the granulation tissue, in infected wounds increased until the 12th day, and then decreased because of commencing maturation of the granulation tissue.

The higher level of ascorbic acid in granulation tissue of infected wounds than of aseptic wounds on the 4th-5th day after the operation (Fig. 1a) can probably be explained by the greater intensity of inflammation, accompanied by predominance of catabolism over anabolism [7]. Under these circumstances the utilization of ascorbic acid for anabolic processes is evidently reduced.

Analysis of the serum ascorbic acid concentration (Fig. 2) showed that in animals with aseptic wounds by the 3rd day of wound healing it fell to 0.82 mg% ($p < 0.05$), but this was followed (on the 7th day) by a marked increase up to 1.2 mg% ($p < 0.001$). By the end of the experiment the serum ascorbic acid level of these animals still remained lower than initially at 0.78 mg% ($p < 0.001$). A similar picture was observed also in animals with infected wounds, although their ascorbic acid level was higher for a longer period of wound healing than in animals with aseptic wounds. This can probably be explained by the more intensive mobilization of ascorbic acid in response to operative trauma and infection, and also, perhaps, by its inadequate utilization in the wound tissues in connection with the predominance of catabolic processes.

The results showing the hydroxyproline concentration in granulation tissue indicated that the time course of its change was similar in character in both aseptic and infected wounds (Fig. 3). Starting with the first time of investigation, for instance, the hydroxyproline concentration in granulation tissue of the experimental animals increased during wound healing. However, quantitatively speaking, the hydroxyproline level in the infected wound was significantly higher than in the aseptic wound.

According to the morphological data, in aseptic wounds granulation tissue matures faster than in infected wounds: fibroblasts differentiate and produce collagen faster, and intensive fibrillogenesis and collagen fiber formation take place. The granulation tissue undergoes more rapid fibrosis and is converted into scar tissue, in which collagen fibers are much more numerous than cells. In infected wounds this process is delayed. We know that less mature connective tissue contains a larger quantity of soluble collagen fractions: salt- and acid-soluble [5]. Immature collagen fibers contain fewer intra- and intermolecular linkages than mature [6].

The higher level of hydroxyproline in granulation tissue of infected wounds can consequently be explained on the grounds that the method used was able to determine hydroxyproline in mainly soluble collagen fractions and in insoluble fractions with a reduced number of intermolecular linkages, i.e., immature collagen.

In infected wounds maturation of collagen and conversion of granulation tissue into fibrous scar tissue are delayed, and it is this which causes delay of wound healing. This delay is manifested both in the time course of the hydroxyproline concentration in the granulation tissue and in the change in the serum ascorbic acid level. These parameters can serve as an objective quantitative criterion for evaluating the state of healing of aseptic and infected wounds and can be used for the prognosis of wound healing and the choice of method of wound therapy.

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EFFECT OF LIGHTING CONDITIONS ON CIRCADIAN RHYTHM OF RECTAL TEMPERATURE IN MICE

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UDC 612.33:612.563].014.44.087"52

KEY WORDS: temperature, natural conditions, continuous light, circadian rhythm, segregation.

One of the principal synchronizers for animals is the alternation of light and darkness, changes in which (a phase shift of lighting, in the length of the period, continuous daylight or darkness, reversal of the lighting conditions) lead to disturbances of the circadian rhythms of physiological processes, including those of an integral factor such as the rectal temperature [2, 4, 6, 9].

All the investigations cited above were undertaken under artificial lighting conditions, as regards both experiment and control. Accordingly, it is highly interesting to compare the circadian rhythm of rectal temperature in nocturnal animals kept under conditions of continuous lighting, and under natural conditions of alternation of day and night, for in that situation daylight and darkness do not begin suddenly, as with a fixed lighting schedule, but gradually, with a period of twilight. Moreover, by continuing the study for a long period of time, it is possible, on the one hand, to discover the principles observed in the character of the temperature rhythm during adaptation to continuous light and, on the other hand, to determine what changes may characterize the temperature rhythm under natural conditions during different lengths of daylight in different months of the year.

The circadian rhythm of rectal temperature was studied in the present investigation for 4.5 months in mice kept in continuous daylight and during natural alternation of day and night.

Department of Biology, N. A. Semashko Moscow Medical Stomatologic Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR, D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 9, pp. 358-360, September, 1988. Original article submitted June 13, 1987.

0007-4888/88/0009-1337$12.50