DISTURBANCE OF OXIDATIVE METABOLISM AS CRITERION FOR EVALUATING THE COURSE OF EXPERIMENTAL WOUND HEALING

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The principal methods of diagnosis of the development of septic inflammatory processes in postoperative wounds in general clinical practice still continue to be: analysis of the results of clinical observation, the data obtained by a general clinical study of the blood, and instrumental, biochemical, immunologic, cytologic, and electrophysiological methods of investigation [6]. These methods often indicate a septic inflammatory process which has already developed in postoperative wounds, when preventive measures have been used late, and when a septic focus has to be treated. The advantages of any diagnostic method are its operativeness and informativeness, which are determined by a detailed study of the function of every system involved in wound healing and the mechanisms of their interaction and damage. One of these mechanisms is a disturbance of the pro- and antioxidant balance and of systems coupled with it, the importance of which, according to several authorities, in ischemic heart disease and atherosclerosis [5], in burns [2], and in aseptic inflammation [10] is beyond dispute.

The aim of this investigation was to develop new ways of diagnosis of the possible development of septic inflammation in postoperative wounds on the basis of a study of oxidative metabolism in experimental animals.

EXPERIMENTAL METHOD

The investigation was conducted on two groups of animals. In group 1, on 90 male Wistar rats weighing 190-200 g, the experimental model consisted of two-dimensional muscular wounds with an area of 400 mm^2, complicated and uncomplicated by infection. The model of a wound uncomplicated by infection was created as follows: under hexobarbital anesthesia (150 mg/kg body weight, intramuscularly), an area of skin with subcutaneous cellular tissue and superficial fascia was removed on the dorsal aspect of the neck of the rats after depilation, with observance of the rules of asepsis and antisepsis, and a Teflon ring was inserted into the wound, which was covered by means of a perforated film. To obtain a model of a wound complicated by infection, the edges and floor of the wound were subjected to additional trauma by dental forceps, and 0.5 ml of a suspension of a 24-h culture of a pathogenic staphylococcus strain 75 A (in a concentration of $1.5 \cdot 10^6$ microbial bodies in 1 ml of physiological saline) was introduced inside the ring. The ring was removed 2 days later. Changes in the pro- and antioxidant systems in the tissues of the wound bed and in the blood serum, and changes in the acid–base balance (ABB) of the blood during the development of septic inflammation in the soft tissues in the first 2 days after the operation were studied. From parameters of the pro- and antioxidant systems in the blood before the operation and in the blood and wound bed tissues 48 h after the operation the specific activity of superoxide dismutase (SOD) was determined by a spectrophotometric method [9, 15]. The level of hydroperoxides was determined by a thermoluminescent method [3], and changes in ABB in the capillary blood were studied by Astrup's method. Tissue samples were taken from the wound bed at the same times and investigated morphologically (staining with hematoxylin and eosin, by Van Gieson's method, etc.). From 10 to 12 animals were used at each time point. The animals were decapitated after the samples had been taken. Methods of diagnosis of the development of septic inflammation in postoperative wounds were developed on the animals

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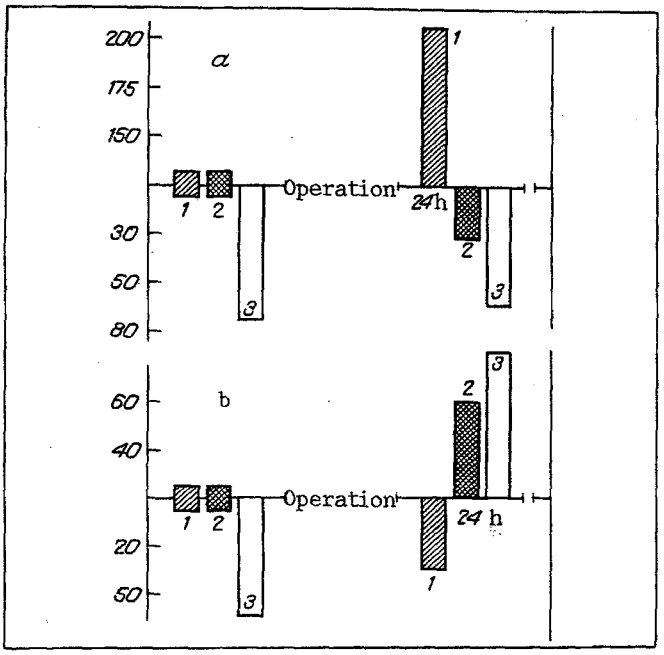

Fig. 1. Changes in hydroperoxide level, specific superoxide dismutase activity, and response to presence of endogenous formaldehyde in blood serum of animals with wound healing uncomplicated (a) and complicated (b) by infection. Abscissa, time of determination of test parameters (in h); ordinate: on left — level of hydroperoxides (1), and specific superoxide dismutase activity (2) in % of intact animals; on right – qualitative reaction for presence of endogenous formaldehyde in blood serum.

of group 2, for which purpose experiments were carried out on 10 chinchilla rabbits weighing 2.5-3 kg, in two series with wounds complicated and not complicated by infection. All the animals were given an intravenous injection of 0.143 mg/kg body weight of a 40% solution of hexamethyltetramine 12 h after the operation. Blood for investigation was taken from the auricular vein before the operation and 24 h thereafter. SOD activity, levels of hydroperoxides, and changes in ABB of the blood (pH) were determined in the blood serum of the animals. The last parameter was determined on the basis of decomposition of hexamethyltetramine in an acid medium into ammonia and formaldehyde by a qualitative reaction [1].

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that during healing of wounds uncomplicated by infection an increase in the hydroperoxide concentration was observed in the tissues of the wound bed during the first 2 days after the operation by 76-80% more than in undamaged tissues, a situation which corresponds to the beginning of the inflammatory phase of wound healing. From the 1st day after the operation morphological investigations revealed degenerative and necrotic changes in cells in the wound floor or even total destruction of their nuclei and cytoplasm. The body as we know responds to the action of various traumatic agents with a marked phagocytic reaction [12], which is accompanied by a burst of respiratory activity with the production of free oxygen radicals (·O, ·OH, O_2^{-}, etc.) [8, 13]. Intensive formation of superoxide radicals, according to data in the literature [12], activates chemotaxis of the leukocytes, and this evidently leads to an increase in their number in the wound, with marked infiltration of the wound edges and floor by neutrophils. Injury to biomembranes of the wound tissue cells disturbs the pro- and antioxidant balance of the cells only a little, as shown by a decrease of 40% in SOD activity and a high hydroperoxide concentration (Fig. 1a). Hydroperoxides, inactivating the sulfhydryl groups of enzymes [11], cause disturbance of vascular permeability with the development of edema and limitation of the focus. Under these circumstances active substances with bactericidal properties are formed [14], depressing growth of microbial colonies in the wounds. One result of these processes is evidently aseptic inflammation and a course of wound healing uncomplicated by infection.

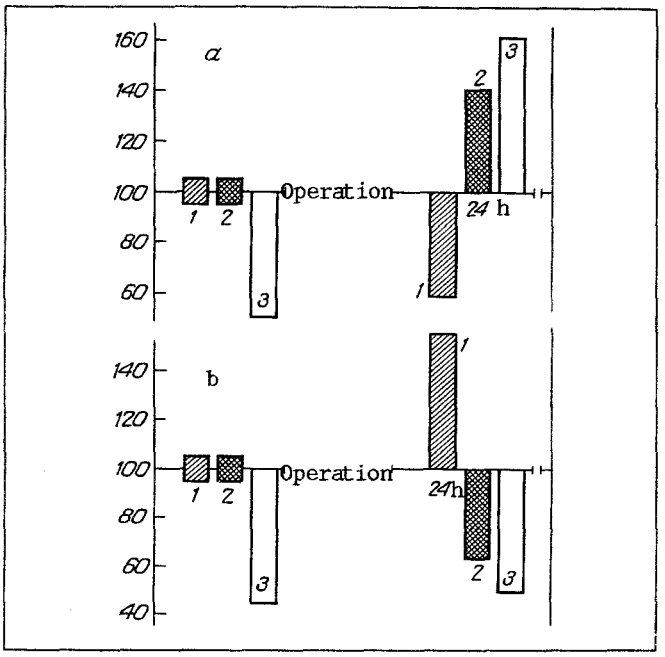

Fig. 2. Changes 24 h after operation in hydroperoxide levels, specific superoxide dismutase activity, and reaction for endogenous formaldehyde in blood serum of rabbits with wound healing uncomplicated (a) and complicated (b) by sepsis. Abscissa, time of determination of test parameters (in h); ordinate: on left – level of hydroperoxides (1), specific superoxide dismutase activity (2), in % of preoperative level of these parameters; on right – result of qualitative reaction for presence of endogeneous formaldehyde in blood serum.

By diffusing together with products of wound metabolism the free radicals cause elevation of the hydroperoxide level (40-100%) and compensatory lowering of specific SOD activity by 10% or more in the blood serum (Fig. 1a), and caused virtually no change in the value of ABB of the blood by comparison with the initial values.

In wounds complicated by infection a different picture was observed at these times: on account of severe damage, destruction of muscle tissue (during creation of the experimental model), hemorrhage, microbial invasion, and more severe trophic disturbances (disturbance of nutrition and necrosis of connective and adipose tissues) and of exudative processes (increased vascular permeability, diapedetic hemorrhages, aggregation of erythrocytes, microthromboses into the lumen of the vessels, edema, and neutrophilic infiltration), compared with the uncomplicated course of wound healing. This situation provokes intensive initiation of peroxidation of the lipid layer of wound tissue and blood cells, the antioxidant protection of the cells and synthesis of certain enzymes are disturbed, and the concentrations of metallic ions (Fe^{2+} and Cu^{2+}), of ceruloplasmin, transferrin, etc., are altered [4]. Response quenching of hydroperoxides is induced, with a fall of their level by 60% and a sharp reduction in specific SOD activity by 30% in the tissues compared with the uncomplicated course. Possibly the body responds to the arrival of products of wound metabolism in the blood stream and to the severity of processes taking place by maximal release of SOD from the depots and disturbance of activity of the buffer system of the blood with the development of acidosis, observations which do not contradict data in the literature [7]. Consequently, SOD activity is increased by 50% and the hydroperoxide level falls by 20-30%.

Thus the basic changes determining the subsequent course of wound healing take place during the first 24 h after wounding, and later the course of wound healing is aggravated and structural changes predisposing to one or other type of course take place. The parameters of the change in pro- and antioxidant balance and in ABB of the blood (pH) are already informative during the first 24 h and can be used as independent tests for diagnosis of the possible development of sepsis.

The results served as a basis for experimental studies of group 2, with the aim of developing new methods of diagnosis of the possible development of sepsis in postoperative wounds 24 h after wounding.

In 10 rabbits the qualitative test for formaldehyde was carried out before the operation in the blood serum, and was negative (an orange color of the samples tested) and their superoxide dismutase activity and hydroperoxide levels also were determined.

In five rabbits in the experiments of series I with wounds uncomplicated by infection, 12 h after the operation a single intravenous injection was given of 0.143 mg/kg body weight of a 40% solution of hexamethyltetramine, and 6-12 h after the injection (18-24 h after the operation) a second blood sample was taken and specific SOD activity, hydroperoxide levels, and the presence of formaldehyde in the blood serum were determined. The qualitative reaction for formaldehyde was negative (the samples stained an orange color). Injection of 40% hexamethyltetramine solution did not affect the change in specific SOD activity and hydroperoxide levels: 20-30% lower and 80-100% higher than the preoperative level of these parameters (Fig. 2b), corresponding to an uncomplicated course of wound healing. Only in one rabbit was partial wound sepsis observed.

In series II, in five rabbits with wounds complicated by infection, preliminary measures also were carried out as in series I. The following changes were observed in the blood serum of these animals 24 h after creation of the model wound: an increase of 40-60% in the specific SOD activity and a fall of 20-50% in hydroperoxide levels (to the preoperative level of these parameters); testing the sera for formaldehyde revealed specific staining (pink), evidence of the presence of formaldehyde in the blood (Fig. 2a). Clinically this was confirmed on the 4th-5th day by the appearance of marked inflammation (swelling of the tissue, hyperemia, and edema), with as a result, cutting out of the sutures, complete separation of the wound edges, and the appearance of a purulent discharge.

Thus by studying disturbances of oxidative homeostasis of the animal during the formation of a septic inflammatory focus in postoperative wounds 24 h after the operation, characteristic changes reflecting this process can be detected, i.e., possible sepsis of postoperative wounds can be predicted in good time. By predicting the course of wound healing, early corrective treatment can be given for a specific purpose and on a logical basis, very important considerations in clinical practice.

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