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TIME COURSE OF LIPID PEROXIDATION AND TISSUE RESPIRATION DURING HEALING OF EXPERIMENTAL ASEPTIC AND INFECTED WOUNDS

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Much research has recently been published to show that reactions of lipid peroxidation (LPO) are intensified in various pathological processes and, in particular, in burns [6], atherosclerosis and ischemic heart disease [2, 3], and aseptic inflammation [14]. Meanwhile its role in the pathogenesis of wound healing, especially when infection is present, has been inadequately studied.

The aim of this investigation was to study LPO during healing of aseptic and infected wounds and to compare changes in LPO activity with the time course of tissue respiration.

EXPERIMENTAL METHOD

Two series of experiments were carried out on 250 male Wistar rats weighing 180-200 g. Full-thickness aseptic and infected wounds with an area of 400 mm^2, inflicted under general hexobarbital anesthesia (150 mg/kg, intramuscularly) on the dorsal aspect of the animal's cervical region, served as the experimental model. After removal of an area of skin with the subcutaneous cellular tissue and superficial fascia, a plastic ring was inserted into the wound and covered with perforated film. To obtain an infected wound, the edges and base of the wound were traumatized by toothed forceps, and 0.5 ml of a suspension of a 24-h culture of a pathogenic staphylococcus (strain 209), containing 1.5×10^9 microbial cells to 1 ml of physiological saline, was introduced inside the ring. The ring was removed on the 3rd day.

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The intensity of LPO was estimated from the concentration of malonic dialdehyde (MDA) [11]. The oxygen consumption of the outer surface of the isolated wound bed was recorded on a PA-2 polarograph (Czechoslovakia) in a special chamber with physiological saline at 37 ± 0.1°C by the method in [12].

The DNA level and the intensity of tissue respiration were investigated at intervals: before the operation (control), and 1, 2, 3, 5, 8, 10, and 15 days thereafter. The serum MDA concentration of intact animals, and also the MDA concentration and rate of oxygen consumption in the tissue (muscle) from the region of the wound of animals immediately after the operations, were used as the control. Serum was obtained by the standard method from blood taken from the femoral vascular bundle. Wound bed tissue weighing 200-300 mg was excised from the same animals after decapitation and the intensity of tissue respiration and the MDA level in it were determined. At each experimental point no fewer than three animals were used in the serum experiments and no fewer than seven in the tissue experiments.

EXPERIMENTAL RESULTS

During the first three days, corresponding to the phase of traumatic inflammation [9], a moderate increase in the MDA concentration took place in the tissue and serum of animals with aseptic wounds. In the animals with infected wounds, the rise of the MDA level was more marked and reached a maximum on the 2nd day. On the 3rd day it declined, and the decrease was particularly marked in tissue. Meanwhile intensive oxygen consumption by the tissue was observed in these animals on the 2nd day, followed by a significant decrease on the 3rd day, unlike this parameter in the animals with aseptic wounds, in which the intensity of respiration was low throughout this period.

The strong activation of LPO in the infected animals was connected with factors of additional trauma and the infection itself. A special experiment showed that on the 2nd day after wounding, either infection alone or trauma alone increased the MDA concentration compared with that in aseptic wounds by 50-60%, and both factors together increased it by almost 150%. These two factors thus have a mutually potentiating action, leading to the development of an acute suppurative inflammatory reaction.

A marked increase in the intensity of respiration in the tissues of the infected wounds on the second day was probably due to increased generation of active forms of oxygen by neutrophils and macrophages migrating into the pathological focus from the blood stream [4, 8, 15]. During active phagocytosis the rate of respiration of these cells may be increased by 10-20 times [5]. Active forms of oxygen are intended mainly for combating microorganisms, but being produced in large amounts, they may emerge into the intercellular space and initiate LPO.

By the 3rd day the oxygen consumption and MDA level in the infected wounds fell considerably. This was evidently due, on the one hand, to the beginning of subsidence of traumatic inflammation and, on the other hand, to activation of the protective antioxidant systems of the body. For example, it has been shown in experimental burns that glutathione peroxidase and superoxide dismutase activity in the liver increases until the 3rd day [6].

During the next period, lasting 8-10 days from the beginning of the operation and corresponding to the proliferative phase of inflammation [9], oxygen consumption by the tissues of both aseptic and infected wounds became more rapid. However, in the former, possibly due to better preservation of tissue function, this process was more marked and reached a maximum by the 8th day, compared with the 10th day in the case of infected wounds. This intensification of respiration was evidently due to the increased number of cells at this period, especially fibroblasts [10], required for granulation tissue formation [9]. It was shown in [1, 13] that the respiration rate of granulation tissue of wounds affecting skin and muscles is maximal on the 7th-10th day, due to an increase in the number of cells and increased activity of respiratory enzymes, which is associated with expenditure of energy on the more intensive biosynthetic processes.

During this period (until the 8th day) the MDA level in the tissue and serum of animals with infected wounds began to rise again. It continued to rise also in the tissues of animals with aseptic wounds, although at a lower level than in the former. A similar time course of accumulation of LPO products was discovered on a model of aseptic granulomatous inflammation in the exudate [14] and in various organs of animals subjected to mechanical asphyxia followed by resuscitation [7]. Stimulation of LPO in aseptic wounds until the 8th day was accompanied at the same time by lowering of the MDA level in the serum of these ani-

mals virtually to normal, evidently due to limiting of the process and to formation of a wound barrier, preventing the release of LPO products into the blood from the pathological focus. Meanwhile, elevation of the serum MDA level of animals with infected wounds indicates a less well developed protective barrier.

Intensification of LPO toward the end of this period (8th-10th day) may be due to exhaustion of the protective antioxidative systems. For instance, in burns and aseptic inflammation, activity of antioxidative enzymes and concentrations of free SH-groups and α-tocopherol in the liver have been shown to be reduced [6, 14]. It is also known that free radicals and LPO products can themselves inactivate enzymes of antioxidative defense [15], and for that reason the more intensive LPO in animals with infected wounds probably leads to greater vulnerability of the antioxidative systems.

Finally, in the 3rd period of wound healing (from the 8th-10th day until the end of observation), characterized by a decrease in cell concentrations [10] and the beginning of maturation of granulation tissue [9], a decrease in the rate of respiration and the MDA concentration was observed in both wound tissues and serum; their levels in infected wounds remained raised, however, even until the 15th day.

The time course of the MDA level and tissue respiration in infected wounds thus differs from that in aseptic wounds. On the whole, the MDA concentration in infected wounds was higher and had two peaks: on the 2nd and the 8th-10th day after wounding. Changes in the serum MDA concentration of rats with infected wounds were similar to those in tissue, but were less marked, whereas the serum MDA level of animals with aseptic wounds differed from its tissue level, and it returned virtually to normal after the 5th day. Respiration of granulation tissue as an indicator of proliferative and biosynthetic activity was more intensive in aseptic wounds. Some degree of similarity will be noted between the time course of oxygen consumption and that of the level of LPO products, and it thus seems that these processes may be interconnected.

The results are evidence that the time course of the intensity of LPO and respiration correlates with the phases of wound healing and depends on its character. These parameters can accordingly be used both to study the mechanisms of wound healing and to develop a method of pathogenetic treatment and to evaluate its effectiveness.

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