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INTERACTION BETWEEN SEROTONIN AND LIPID PEROXIDATION PRODUCTS DURING EXPERIMENTAL WOUND HEALING

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Wound healing takes place in definite stages [6, 9]. The most complex changes (at tissue, cellular, and molecular levels) take place in stage I of wound healing, in the traumatic inflammation phase. The early manifestations of traumatic inflammation are due to the involvement of biogenic amines, monoamine oxidase (MAO), free oxygen radicals, and other biologically active substances [1, 5, 10, 11], which determine the abundance and intensity of this reaction and the possible development of a suppurative-inflammatory process [4].

The study of changes in biologically active substances and their inhibitors during wound healing may help with the discovery of ways of preventing wound infection and of stimulating repair processes. This investigation was devoted to the study of this problem.

EXPERIMENTAL METHOD

Experiments were carried out on 220 male Wistar rats weighing 190-120 g. The experimental model consisted of full-thickness aseptic and infected skin wounds covering an area of 400 mm^2, created under sterile conditions by the method described previously [7]. Blood was taken from the femoral vessels of the rats under hexobarbital anesthesia.

Before the operation (initial data) and on the 1st-10th, 12th, and 15th days thereafter, the total serotonin concentration was determined in the blood by a fluorometric method [8]; concentrations of hydroperoxides were determined in the serum and tissues of the wound defect spectrophotometrically [12], and the total protein concentration was determined by the biuret method [16]. To determine the parameters 8-10 animals were used at each experimental point, and after sampling of the tissue, they were killed by decapitation. Samples of wound tissues at the same times were subjected to morphological examination (staining with hematoxylin and eosin, by Van Gieson's method, and with toluidine blue by Brachet's reaction, and various signs of inflammation and repair were evaluated, including the intensity of degranulation and lysis of mast cells (on a 5-point system).

Experiments to study the effect of an MAO inhibitor on wound healing were conducted in two series on 34 male Wistar rats with experimental linear wounds, produced by the method described previously [7], without observance of the rules of asepsis and antisepsis, and with

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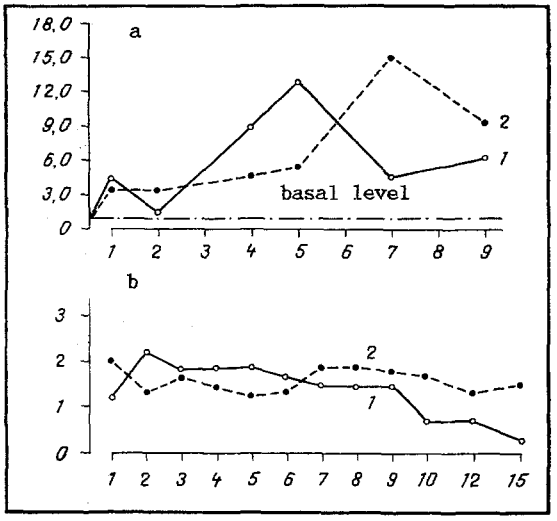

Fig. 1. Blood serotonin concentration (a) and intensity of degranulation of mast cells (b) during healing of aseptic (1) and infected (2) wounds in rats. Abscissa, time after operation, days; ordinate: a) serotonin concentration, relative units; b) intensity of degranulation of mast cells, points.

increased traumatization. In the experiments of series I, immediately after the operation the animals were given 2-isonicotinoyl hydrazide through a special tube per os in a single dose of 20 mg/kg. In the experiments of series II the animals were untreated.

The effectiveness of treatment with the preparation was assessed on the basis of superoxide dismutase (SOD) activity [13] and the level of hydroperoxides 1 day after the operation, and the stimulating action of repair processes was judged by the breaking strength of the postoperative scars on the 7th day after the operation and the number of suppurative complications in the postoperative period.

EXPERIMENTAL RESULTS

The results (Figs. 1 and 2) show that levels of serotonin in the blood and hydroperoxides in the tissues and blood serum were raised in animals with aspetic wounds on the 1st day after the operation. This rise was connected with the release of serotonin from mast cells and platelets into the wound as a result of their mechanical destruction or damage to their biomembranes by active forms of oxygen and by hydroperoxides formed during trauma, for the body responds to the action of trauma by a strong phagocytic reaction [6, 9, 10], accompanied by a "burst" of respiratory activity, leading to initiation of lipid peroxidation (LPO) in the cell membranes. Under these circumstances the released serotonin and the activated MAO, in conjunction with other biologically active substances (histamine, O_2^-, H_2O_2, OH^-, etc.), induce a disturbance of vascular permeability, with the development of edema, etc.

The second rise of the blood serotonin concentration in animals with aseptic wounds began on the 3rd day and reached a maximum on the 5th day (Fig. 1a), after which it fell again. Meanwhile the hydroperoxide level in the wound tissues of these animals fell after the 3rd day (Fig. 2a), but the hydroperoxide level in the blood serum of these animals gradually increased toward the 10th day.

In animals with aseptic wounds this picture can evidently be explained by the kinetics of local processes in the damaged tissues; increased lysis and degranulation of the mast cells (Fig. 1b) observed in the histochemical study from the 2nd through the 6th day led to accumulation of serotonin in the tissues, where it evidently could both participate in the triggering mechanism of proliferation and depress the activity of proliferation inhibitors (chalones [5], for example), leading to stimulation of proliferation, improvement of the microcirculation, and release of products of wound metabolism, biogenic amines, hydroperoxides, etc.) into the blood stream (Figs. 1a and 2b).

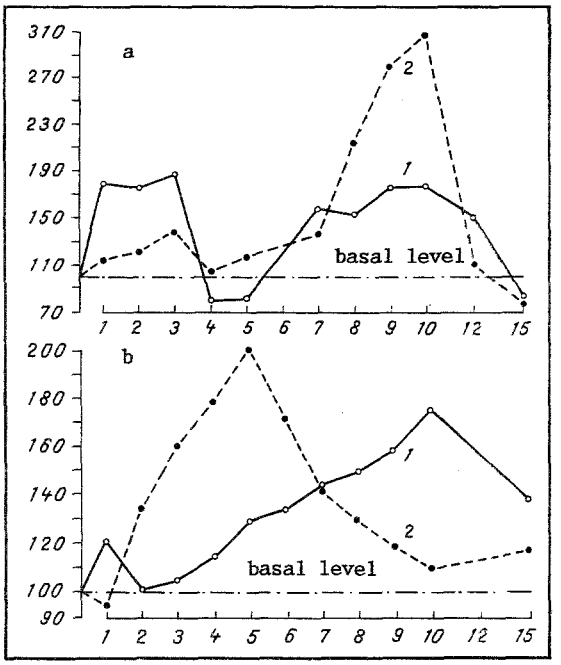

Fig. 2. Level of hydroperoxides in wound tissues (a) and blood serum (b) of rats with aseptic (1) and infected (2) wounds. Abscissa, the same as to Fig. 1; ordinate, hydroperoxide level, %.

Accumulation of biogenic amines toward the 5th day in the blood of these animals potentiates activity of the serotonin-utilizing systems: cytochrome [2], ceruloplasmin [14], and mitochondrial membrane MAO [2]. The last of these enzymes catalyzes the oxidative deamination of free serotonin to its end product, namely 5-hydroxyindoleacetic acid, during which peroxides accumulate, and, if catalase activity is insufficient, they form highly reactive hydroxyl radicals (OH^-) by Fenton's mechanism. The latter can initiate LPO (Fig. 2a). Products of LPO and phagocytosis (OH^-, O_2^-, H_2O_2, etc.) are utilized by the antioxidant system, which gives rise to a very small rise in the tissue hydroperoxide level (Fig. 2a) and to their gradual accumulation in the blood (Fig. 2b), which is compensatory in character. A marked increase in the hydroperoxide level was observed in the blood of animals with infected wounds (Fig. 2b) toward the 5th day, but an increase in the serotonin concentration was not observed until the 7th day. Meanwhile, from the 3rd through the 7th days, a low level of hydroperoxides was observed in the wound tissues of these animals, followed by a sharp rise on the 10th day (Fig. 2a).

Active initiation and catalysis of LPO by free oxygen radicals and Fe^{++} ions formed during phagocytosis by oxidative deamination of serotonin, destruction of blood and tissue cells, and predominance of a fibrino-purulent exudate, promote intensive accumulation of hydroperoxides. Active forms of oxygen exhaust the protective potential of the antioxidant system, act destructively on surrounding tissue cells [4], and promote reciprocal inhibition of activity of MAO and qualitative modification of its properties [3], thereby raising the serotonin level and disturbing collagen synthesis. This state of affairs can evidently explain the prolonged inflammation (compared with aseptic), which was revealed by histological investigation, and the more prolonged healing of infected wounds (the average time of healing of infected wounds was 29.5 ± 0.4 days, and of aseptic wounds 23.5 ± 0.5 days). This situation is decompensatory in character.

Comparative analysis of the change in the blood serotonin concentration in animals with aseptic and infected wounds thus suggests that during the development of suppurative inflammation, on account of oxidative deamination of endogenous biogenic amines LPO is activated; this process, taking place in the immediate microenvironment of membrane-bound MAO, reduces MAO activity and causes subsequent inhibition of the antioxidant system. On the whole this evidently "distorts" the course of inflammation.

To create more physiological conditions for the course of inflammatory repair processes in postoperative wounds, the endogenous serotonin level is therefore best raised and the level

TABLE 1. Effect of 2-Isoicotinoyl Hydrazide on Wound Healing in Rats (M ± m)

Experimental conditions	1st day				7th day				
	SOD, specific activity units/mg protein		Hydroperoxides, nmoles/mg		10th day number of suppurative foci		character of healing		Strength of scar, mg/mm
	initial data	after 24 h	initial data	after 24 h	partially	completely	primary	secondary	
Control (n = 14)	3,83±0,27 (6)	4,95±0,2 (6)	0,045±0,002 (6)	0,032±0,002 (6)	2 (8)	6 (8)	0 (8)	8 (8)	26,8±4,2 (8)
Injection of 2-isonicotinoyl hydrazide (n = 20)	3,83±0,27 (10)	2,6±0,1 (10)	0,045±0,002 (10)	0,088±0,004 (10)	1 (10)	1 (10)	8 (10)	2 (10)	41,8±2,3 (10)

Legend. Number of animals given in parentheses.

of LPO products lowered. This problem cannot be solved by introduction of exogenous serotonin into the wound, for this is very painful and induces an extremely strong vascular reaction, manifested by exudation and subsequent disturbance of collagen synthesis [15]. This can be avoided by the use of stimulators of biogenic amine metabolism and, in particular, of MAO inhibitors.

Injection of 2-isonicotinoyl hydrazide into the rats immediately after the operation led to wound healing in the animals without any marked inflammatory phenomena (by primary intention). On the 7th day, a thin elastic scar was formed in the region of the wound defect, with a strength of 51.8 ± 2.3 mg/mm^2. In our untreated animals the wounds discharged pus, and in six they healed by first intention (Table 1), although the inflammatory phenomena were more marked in degree, during both microscopic and histologic study, and the strength of the scar was 26.0 ± 1.2 mg/mm^2.

The positive action of 2-isoicotinoyl hydrazide can be explained by increased antioxidant activity, rapid utilization of free radicals and hydroperoxides, and maintenance of their optimal level for the normal course of wound healing. This is confirmed by the fact that 24 h after the operation (Table 1), specific SOD activity and hydroperoxide concentrations showed a return to normal, compared with the control, and this may have modified the character of inflammation.

The degree of destruction of relations between the pro- and antioxidant system, biogenic amines, and their utilizing systems determines the character of inflammation during wound healing. Consequently, to prevent and abolish peroxide-induced cell damage in the course of wound healing, it is evidently worthwhile to use, not only antioxidants, but also highly effective selective MAO inhibitors. Treatment of suppurative wounds in the second phase of wound healing by preparations inactivating serotonin or potentiating its utilization may be beneficial.

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LIPID PEROXIDATION AND POLYMERASE ACTIVITIES OF LIVER CHROMATIN FRACTIONS OF AGING RATS

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The aging process is accompanied by definite changes in structure and function of the cell genome [8]. The writers previously demonstrated slowing of replication and transcription processes and changes in the distribution of DNA-polymerase activity in subcellular fractions, and also of certain structural properties of the liver chromatin of rats in [9, 10, 12].

Replication and transcription processes in the cell nucleus are located in the macromolecular protein–nucleic acid–lipid chromatin complex, which is the structural and functional form of organization of the nuclear genome. Endogenous DNA- and RNA-polymerase activities of chromatin determinable in vitro must evidently correlate with the intensity of replication and transcription processes in vivo.

Lipids, which are components of the nuclear genetic apparatus, can evidently perform regulatory functions [1]. In particular, the phospholipid sphingomyelin, which destabilizes the DNA double helix, can activate replication and transcription processes [2]. It has also been suggested that lipid peroxidation (LPO) of chromatin [1, 5] may be one factor regulating replication and transcription processes when disturbed during aging. The possibility therefore cannot be ruled out that disturbance of the regulatory effect of peroxidized chromatin phospholipids on its function may be one cause of changes observed in function of the nuclear genome during aging.

The aim of this investigation was to study relations between the intensity of NADPH- and ascorbate-dependent LPO and activities of DNA- and RNA-polymerases in fractions of actively transcribed and repressed liver chromatin of mature and old rats.

EXPERIMENTAL METHOD

Male Wistar rats aged 8 months (mature, 200-300 g) and 26 months (old, 300-400 g) were used. The animals were decapitated under superficial ether anesthesia during the morning hours, the liver was removed from them and preparations of actively transcribed and repressed chromatin were isolated from it [10]. DNA- and RNA-polymerase activities were determined by the methods in [6, 11]. Activities of DNA-polymerases α and β were separated on the basis of their differential sensitivity to N-ethylmaleimide, and activities of RNA-polymerases I and II on the basis of their differential sensitivity to α-amanitine. LPO of chromatin (NADPH- and ascorbate-dependent: NDP and ADP, respectively) was assessed on the basis of

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