PLASMA SERINE PROTEASE ACTIVITY DURING HEALING OF EXPERIMENTAL ASEPTIC AND INFECTED WOUNDS

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KEY WORDS: kallikrein-like activity; plasmin-like activity; plasma; wound.

An increase in the number of postoperative suppurative complications and their inadequately effective treatment constitute one of the most acute problems in modern surgery [6, 9, 10]. The course of suppurative inflammatory postoperative diseases is based on complex biological processes, in which an important role is played by proteolytic enzymes: serine proteins of the trypsin type (including plasmin and kallikrein) and their inhibitors. Thrombin, kallikrein, plasmin, and their activators and inhibitors belong to a single factor XII-dependent protective system of the blood [11]. The coordinated action of individual components (kinin formation, fibrinolysis, clotting) of this system is essential for maintenance of the optimal ratio between the rheologic properties of the blood and vascular tone and permeability [2, 13]. Dynamic changes in the relative activities of individual enzymes of this system can be observed both during physiological conditions of adaptation and in various pathological states. In some cases these changes are insufficient for restoration of normal functions or they are harmful in character, and necessitate administration of drugs to correct activity of the enzymes of the factor XII-dependent system. The contradictory nature of data in the literature on the indications for use of these therapeutic substances in the treatment of suppurative wounds [3, 6, 12] makes it impossible to determine in what concrete cases proteolytic enzymes of their inhibitors should be given.

The aim of this investigation was to study the role of serine proteases in healing of aseptic and infected wounds.

EXPERIMENTAL METHOD

Two series of experiments were carried out on 220 male Wistar rats weighing 200-210 g. Aseptic and infected superficial 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 [7]. To obtain a model of an infected wound, the edges and floor of the wound were additionally

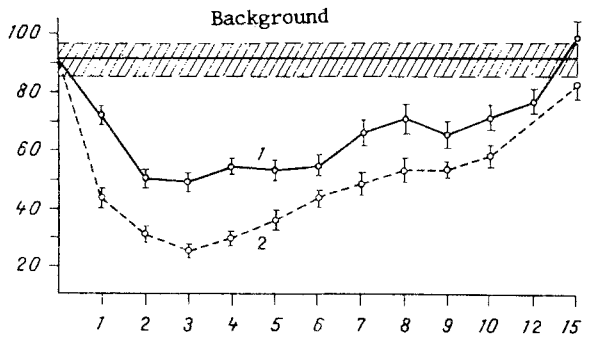

Fig. 1. KLA in blood plasma during healing of aseptic (1) and infected (2) wounds in rats. Abscissa, time after operation (in days); ordinate, KLA activity (in U/liter).

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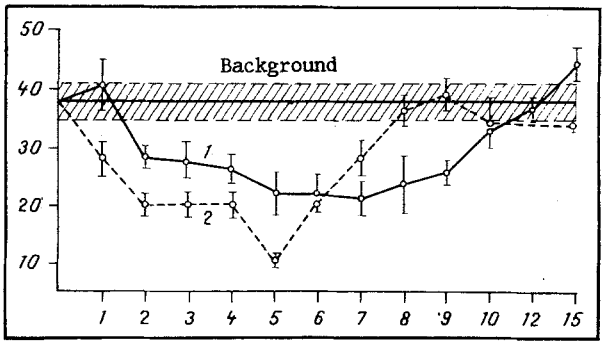

Fig. 2. PLA in blood plasma during healing of aseptic (1) and infected (2) wounds in rats. Abscissa, time after operation (in days); ordinate, PLA (in U/liter).

traumatized with toothed forceps, and 0.5 ml of a suspension of a 24-h culture of pathogenic staphylococcus (1.5×10^9 bacterial cells/1 ml physiological saline) was introduced into the wound surface. The kallikrein-like and plasmin-like activities (KLA and PLA respectively) in the blood plasma were determined with the use of chromatogenic peptide substrate before the operation, and daily from the 1st through the 10th days and also on the 12th and 15th days after the operation. For analysis of enzyme activity 50 µl of citrated plasma was diluted in 500 µl of 50 mM Tris-HCl buffer (pH 7.4 or 7.8). A 100-µl sample of diluted plasma was treated with 100 µl of a 2 mM solution of the substrate and then incubated at 37°C for 17 min. The enzyme reaction was stopped by the addition of 300 µl of 50% acetic acid. The experimental samples were subjected to photometry against a corresponding control at a wavelength of 405 nm. Meanwhile, at the times indicated above, aspartate aminotransferase (AST) activity was determined on an LKB-2086 reaction velocity analyzer (Sweden) in the serum of these animals by kinetic methods [16], and the number of leukocytes was counted in the blood. At each time point 8-10 rats were used and killed by decapitation. The numerical data were subjected to statistical analysis.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that during healing of aseptic and infected wounds the values of KLA were significantly below the background levels. Despite the similarity of the curves reflecting changes in this parameter in the two series, a significant difference was observed at all times between them ($p < 0.001$): whereas in aseptic wounds the fall in the KLA level was 20-45%, in infected wounds it was 50-74%. The first trough of the decline (by 44%) was observed on the 2nd-4th days after the operation in animals with aseptic wounds and on the 2nd-6th days in rats with infected wounds (by 71%). The peak of a relative rise of the depressed KLA level occurred on the 6th-8th days. By the 12th-15th days the KLA level in animals with aseptic wounds was back to normal, whereas in animals with infected wounds this parameter remained depressed.

PLA in animals with aseptic wounds was reduced by 25-38% ($p < 0.001$) on the 2nd-9th days, and thereafter returned to normal on the 10th-15th days. In animals with infected wounds a significant fall of this parameter ($p < 0.001$) was observed during the first 7 days after the operation, and this was followed by gradual normalization by the 8th day (Fig. 2). Characteristically a trough of decline of both PLA and KLA was observed on the 2nd-6th days. Since the kallikrein–kinin and fibrinolytic systems of the body are closely interconnected [1], it is interesting to correlate these parameters. From the 1st through the 6th day the KLA/PLA ratio changed virtually identically in the animals of both series, but starting with the 7th day in rats with aseptic wounds the KLA/PLA ratio was higher than in the control or the same in value, whereas in animals with infected wounds it was lower (Fig. 3).

The relationship between enzyme activity in aseptic and infected wounds enables an objective assessment to be made of the reactions of the body in response to operative trauma and wound infection. Changes in KLA and PLA in the animals of both series point to a decrease in activity of the kallikrein–kinin and fibrinolytic systems during the first days of would healing. This fact contradicts the accepted concept that these systems are activated during inflammation, and several possible explanations of these contradictions may now be put forward: since KLA and PLA were determined in peripheral blood, the changes discovered do not reflect processes taking place actually in the focus of inflammation (in the wound). It can be

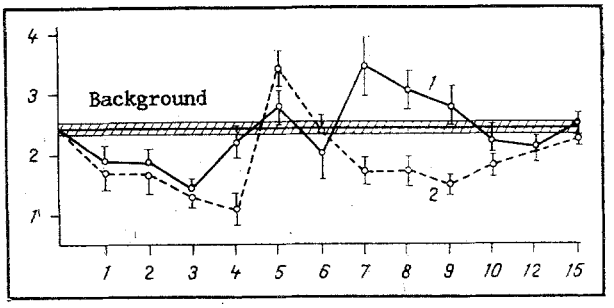

Fig. 3. Changes in KLA/PLA ratio during healing of aseptic (1) and infected (2) wounds in rats. Abscissa, time after operation (in days); ordinate, ratio KLA/PLA.

tentatively suggested that in response to hyperproduction of active proteases in the wounds, their decline and their inactivation in the peripheral blood are intensified. The observed decrease in activity of the kallikrein–kinin and fibrinolytic systems during the first days after wounding may perhaps be the result of hyperactivity of these systems in the first few hours, followed by their exhaustion [1, 5].

The toxic products formed during wound healing lead to a disturbance of liver function and to depression of formation of kallikrein and plasmin precursors. This hypothesis is confirmed indirectly also by the results of our investigations of the serum levels of AST in these same groups, indicating a fall of this parameter during the first days after formation of aseptic and infected wounds.

On the whole the changes in KLA and PLA correlated with several parameters of wound healing. In particular, the lowering of KLA and PLA on the first day after the operation in series with aseptic and infected wounds correlated with migration of the blood leukocytes, containing kinin-forming enzymes [1, 5], and their infiltration into the damaged tissues, leading to increased permeability of the vessel walls, migration of granulocytes into the wound, strengthening of phagocytosis [3], and increased signs of inflammation in the wound. Starting with the 4th day, when the intensity of the suppurative inflammatory processes in infected wounds begins to diminish, destruction of the tissues and leukocytes during phagocytosis was reduced and KLA gradually returned to normal.

Plasmin, another member of the factor XII-dependent enzyme system, regulates the destruction of fibrin and may thus participate in the formation of an inflammatory focus [3, 4, 14]. The lowering of PLA during the first days of the inflammatory reaction was probably connected with the need to restrict the process and to form a "wound barrier," for which the formation of resistant fibrin-containing structures was necessary. Inhibition of activity of this enzyme, which destroys fibrin, is evidently biologically advantageous under these circumstances. The mechanism of inhibition of PLA during the first days of inflammation is not completely clear. Changes in PLA may perhaps be brought about by the same factors as those which cause changes in KLA.

Normalization of the PLA level from the 6th day after the operation corresponds to the period of cleansing of the suppurative wound and the appearance of granulation tissue [8].

The KLA/PLA ratio shows that during the first 5 days changes in enzyme activity in animals with aseptic and infected wounds were virtually identical (Fig. 3). Later, however, this parameter returned to normal in the animals with aseptic wounds, whereas in the infected animals it remained below normal until the 15th day.

It can thus be postulated that wound infection causes more profound changes in the regulatory systems than aseptic wounds. The biochemical "remission" clearly lags behind the clinical remission.

The results point to an active role of factor XII-dependent systems in the pathogenesis of wound healing. However, no concrete recommendations on the use of particular drugs at different periods of wound healing can be formulated on their basis. The fall in activity of the enzymes tested may perhaps be compensatory in character, so that the use of protease inhibitors during the first postoperative days may be justified, and the early fall of PLA on the first day after the operation may be a prognostic sign of the onset of suppurative postoperative complications.

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INTERACTION BETWEEN LABELED ESTROGENS AND RECEPTORS IN HUMAN UTERINE CYTOSOL

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The composition of the estrogen-binding system in the mammalian uterus remains a matter for debate. There is evidence that uterine cells contain estrogen-binding components of several types [8, 11, 12]. At the same time it has been shown [2-4] that rat uterine cytosol contains a single macromolecular form of estrogen receptor. The results of investigations undertaken in the authors' laboratory [1, 7] demonstrated the homogeneity of the estrogen receptor population in uterine cytosol of guinea pigs, rats, and monkeys.

The aim of this investigation was to assess the composition of the estrogen-receptor system of the human uterus by analysis of data relating to interaction between various labeled estrogens and this system.

EXPERIMENTAL METHODS

Uterine cytosol from postmenopausal women was used as the test object because of the low endogenous estradiol content [9]. The cytosol was obtained as described previously [5]. The ratio of weight of tissue to volume of buffer was 1:4. The following reagents were used. Tritium-labeled estrone, estradiol, ethinylestradiol, and estriol, with specific activity of 88,

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