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THE USE OF THERMO- AND ACID-STABLE PROTEINASE INHIBITOR TO DEPRESS PROTEOLYSIS ACTIVATION DURING INFLAMMATION OF THE LUNGS

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The development of an imbalance in the proteinase–inhibitor system is an important pathogenetic factor in the development of inflammatory diseases of the lungs [1, 5, 13]. For this reason an intensive search is in progress, aimed at developing methods of treatment of lung diseases with the use of various preparations of proteinase inhibitors [10, 12, 14]. Among the most effective inhibitors are acid-stable proteins [7], one typical representative of whom is the thermostable and acid-stable inhibitor from rabbit blood serum (TASPI). This inhibitor is a close structural homolog of the acid-stable inhibitors from human blood plasma and urine [8], and its ability to inhibit cellular cathepsin D, elastase, kininogenase, and other proteinases of granulocytes has been described in detail [4, 6]. Special interest has been shown in this class of proteins in connection with the possibility of their clinical use [9].

The aim of this investigation was to compare the efficacy of TASPI, obtained from rabbit serum, as an inhibitor of activation of proteolysis during initiation of inflammation in the lungs with that of the trypsin inhibitor contrykal.

EXPERIMENTAL METHOD

Experiments were carried out on 65 Wistar rats weighing 200 g. Inflammation was initiated by introducing a capron thread 0.25 mm in diameter and 2.5-3 cm long into the animals' trachea. The animals were divided into the following groups: 1) control (healthy animals, n = 21); 2) rats developing inflammation for 24 h (n = 12); 3) rats receiving contrykal before fixation of the thread in the trachea and investigated 24 h later (n = 6); 4) similar to group 3, but receiving TASPI instead of contrykal (n = 6); 5) rats developing inflammation for 48 h (n = 8); 6) rats receiving contrykal 24 h after initiation of inflammation and studied 48 h later (n = 6); and 7) a group similar to

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TABLE 1. Effect of Inhibitors of Proteolytic Activity and Antiproteinase Potential of Rat Blood Following Initiation of Inflammation in the Lungs

Group	n	BAEEea, nmoles/ml·min	α_1-IP, IU/ml	α_2-MG, IU/ml	TASPI, IU/ml
1-st (control, healthy)	21	171,0±18,5	26,7±1,5	3,7±0,2	8,1±0,5
2-nd (inflammation 24 h)	12	239,9±5,5*	37,1±2,5*	1,9±0,1*	8,3±0,3
3-rd (inflammation 24 h + contrykal)	6	188,4±20,5**	28,2±2,5**	2,2±0,3	8,9±0,5
4-th (inflammation 24 h + TASPI)	6	173,0±27,3**	29,9±1,4	2,3±0,7	7,8±0,7
5-th (inflammation 48 h)	8	274,6±28,7*	43,1±1,4*	3,3±0,2	6,2±0,4*
6-th (inflammation 48 h + contrykal)	6	231,4±17,2	36,4±2,4***	4,3±0,3***	6,9±0,6
7-th (inflammation 48 h + TASPI)	6	222,8±38,3	36,7±1,2***	3,2±0,1	7,3±0,3

Legend. *) Significance of differences compared with control, **) compared with group 2, ***) compared with group 5.

group 6, but receiving TASPI instead of contrykal (n = 6). The inhibitors were administered to the experimental animals by the endobronchial route in a dose of 500 antitrypsin units (ATrU)/100 g body weight, TASPI in a dose of 0.5 mg/100 g body weight in 0.1 ml physiological saline. The animals were killed under ether anesther by exsanguination. Bronchoalveolar washings (BAW) were obtained by washing out the lungs 5 or 6 times with 10 ml of 0.9% NaCl solution. Esterase activity of trypsin-like proteinases (BAEEea), activity of proteinase α_1-inhibitor (α_1-IP) and α_2-macroglobulin (α_2-MG). and the level of TASPI in the blood serum were determined [2, 3]. Cells in the BAW were counted in a Goryaev's chamber, protein was determined by Lowry's method, and trypsinlike activity (TLA) and elastaselike activity (ELA) also were determined [5]. Free antitryptic activity (ATA) and the level of acid-stable inhibitors (ASI) were estimated by their ability to bind trypsin [3, 5]. Statistical analysis of the data was carried out by Thomson's and Student's tests. The TASPI used in the study was obtained from rabbit blood serum and its specific activity was 44 IU/mg [2]. The commercial preparation "contrykal" had activity of 48 IU/1000 ATrU, indicating 1000 ATrU of contrykal corresponds to 1 mg TASPI, with respect to antitryptic activity. One inhibitory unit (1 IU) corresponds to an amount of inhibitor required to inhibit hydrolysis of 1 μmole substrate under standard conditions.

EXPERIMENTAL RESULTS

The experiments showed that during the first two days typical acute phasic changes in the proteinase inhibitory system took place in the blood serum (Table 1). TLS rose significantly, a marked rise of α_1-IP was observed, α_2-MG activity fell, and after 48 h TASPI activity also fell. Endobronchial administration of the inhibitor inhibited the response of the blood serum components. During the first day there was virtually no increase in antiproteolytic activity of α_1-IP, but after 48 h the acute phasic rise of α_1-IP was significantly less than without the use of the inhibitors.

Characteristic changes were observed in the study of bronchoalveolar secretion (Fig. 1). During the first two days of development of the response there was an abrupt five-sixfold rise of the protein concentration and a three- to fourfold rise of the cell content of the washings. The degree of saturation of the washings with trypsinlike proteinases increased on account of an increase in protein, although their specific activity did not increase. Meanwhile, the degree of saturation of the washings with elastaselike proteinases increased 12-14-fold, and their specific activity was 3-4 times higher than in the control. In this potentially antiproteolytic state, there was a noteworthy increase in the ability of the washings to bind trypsin and of the α_2-MG level to rise.

Endobronchial administration of both inhibitors led to complementary changes in the reaction of the proteinase-inhibitory potential in response to lung damage. A significantly smaller rise of the protein level and a tendency for the content of cell components in BAW took place. The concentration of trypsinlike enzymes in the washings and their specific activity fell. Elastaselike activity rose by a significantly lesser degree, and when tested after 48 h the ETA values were not significantly higher than in healthy animals.

Despite the complexity of the changes detected in the proteinase–inhibitory system, some special features of the action of TASPI must be mentioned. These include preservation of high values for the number of cells in the washings, the more marked inhibition of activation of trypsinlike and elastaselike proteinases, and an increase in the

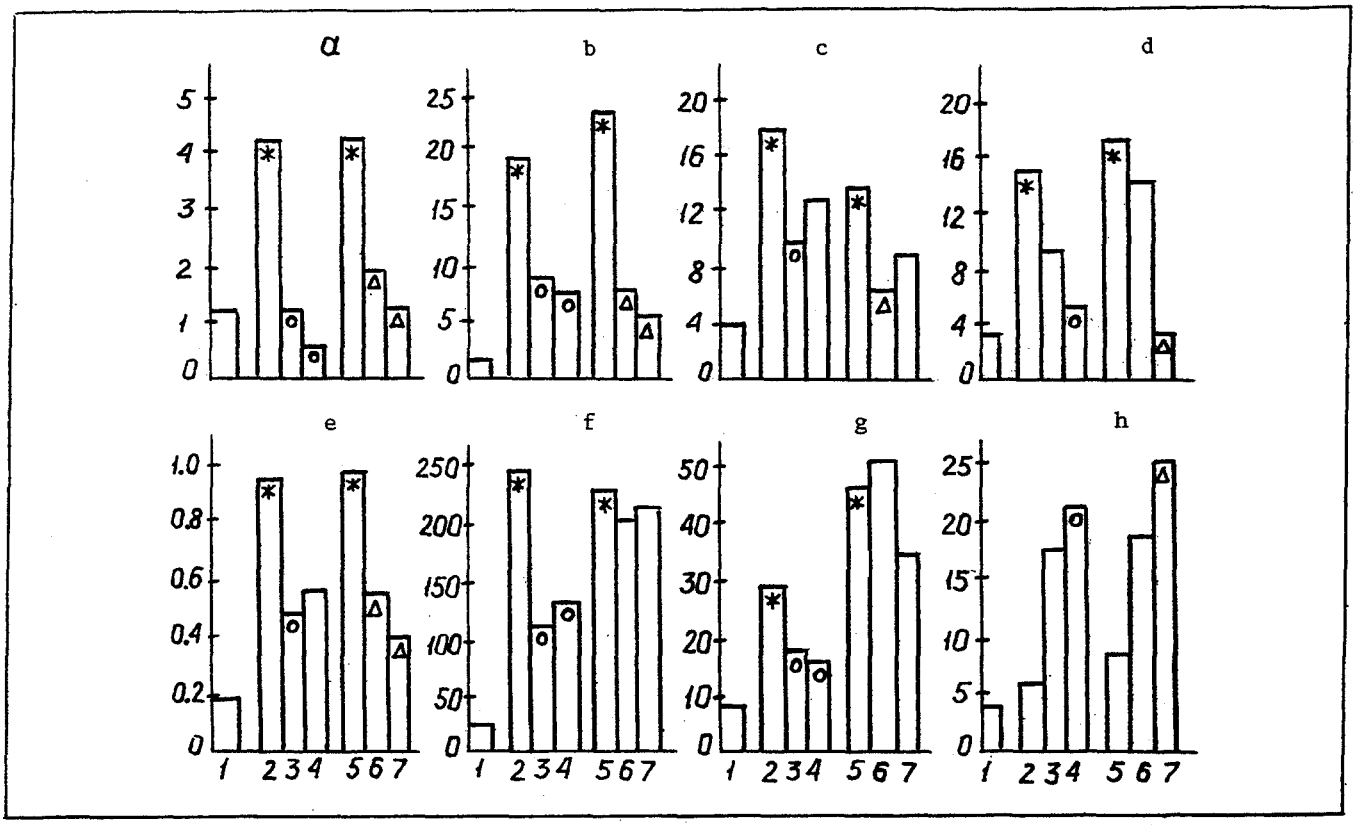

Fig. 1. Dynamics of changes in component of proteinase-inhibitory system of BAW in response to contrykal and TASPI. a) TLA (in nmoles/ml·min), b) ELA (in nmoles/ml·min), c) cells (10^5/ml), d) ELA/cells ratio (in nmoles·10^6), e) protein (g/liter), f) ATA (mIU/ml), g) α_2-MG (mIU/ml), h) ASI (mIU/ml). 1) Healthy animals, 2) Development of inflammation for 24 h, 3) Development of inflammation for 24 h + contrykal, 4) Development of inflammation for 24 h + TASPI, 5) Development of inflammation for 48 h, 6) Development of inflammation for 48 h + contrykal 24 h later, 7) Development of inflammation for 48 h + TASPI 24 h later. *) significance of differences ($p < 0.05$), compared with healthy animals (group 1); ○) compared with group 2; △) compared with group 5.

content of acid-stable inhibitors in BAW. Characteristically, this feature appeared during analysis of the relationship between ELA and the number of cells in the washings (Fig. 1b). When contrykal was used, the decrease in the ratio of ELA to number of cells in BAW was not significant, whereas when TASPI was used, the ELA/cells ratio virtually reached the level found in healthy animals. It can be tentatively suggested that contrykal exerts its main action by preventing infiltration of the bronchoalveolar space by cells, whereas TASPI inhibits more strongly the increasing activity of the free proteinases of the bronchoalveolar secretion.

These features of the action of TASPI may be linked with the different effects of the inhibitors on different groups of proteinases [4, 7, 11]. We undertook additional investigations in vitro to determine the ability of contrykal and TASPI to inhibit activity of elastaselike enzymes. For these investigations pancreatic elastase with specific activity of 0.5 μmole BANPE/min·mg and a pool of bronchoalveolar secretion from rats with mean specific activity of 22.7 ± 0.7 nmole/min·mg protein were used. The experiments showed that the ability of TASPI to inhibit pancreatic elastase was 1110 ± 230 mIU/mg and the equivalent quantity of contrykal was able to bind 13 times less than the elastase (84 ± 15 mIU/1000 ATrU). The ability of the inhibitors studied to depress the elastase activity of the bronchoalveolar secretion in vitro differed by a lesser degree, but TASPI was more than twice as active an inactivator of the elastases of the washings (11 mIU/mg compared with 5 mIU/1000 ATrU for contrykal). The changes discovered confirm the special features in the action of different groups of inhibitors. The affinity of inhibitors of the TASPI type for intracellular proteinases is higher than that of inhibitors of the Kunitz type, as is shown by the more effective action of TASPI on inhibition of cellular proteinases during initiation of inflammation.

Inhibitors of proteolytic enzymes thus effectively depress proteinase activation during the development of inflammation in the lungs. The further study of the possibility of the clinical use of acid-stable inhibitors which, because of their stability and high antielastase activity, may be effective in the treatment of bronchopulmonary diseases, would seem to be promising.

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EFFECT OF PLATELETS ON GENERATION OF ACTIVE FORMS OF OXYGEN BY LEUKOCYTES

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Active forms of oxygen (AFO), including the superoxide anion-radical $O_2^{\cdot-}$, the hydroxyl radical $OH^{\cdot}$, singlet oxygen $^1O_2$, and hydrogen peroxide, generated by leukocytes, play an important role in the development of several pathological processes [5, 10].

AFO realize their pathogenic action through the initiation of free-radical lipid peroxidation (LPO) and by their direct action on cell components and the intercellular substance (collagen, hyaluronic acid) [9].

During a study of AFO generation by leukocytes under normal and pathological conditions [3, 4, 7] we observed that addition of platelet-deprived plasma (PDP) to the leukocytes stimulates AFO generation by a greater degree than addition of platelet-enriched plasma (PEP). This observation evidently indicated that platelets can influence the stimulating action of plasma on AFO generation by leukocytes. We found a report in the literature of the ability of plasma to stimulate AFO generation by leukocytes [2], but the role of platelets was not analyzed. Only

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