Proteinases in Inflammation and Tumor Invasion

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PROTEINASE INHIBITOR THERAPY OF SEVERE INFLAMMATION IN PIGS: FIRST RESULTS WITH EGLIN, A POTENT INHIBITOR OF GRANULOCYTE ELASTASE AND CATHEPSIN G

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Introduction

Lysosomal enzymes released during inflammation may enhance the inflammatory response by specific activation of humoral systems (clotting, fibrinolysis, complement, kallikrein/kinin system) or by unspecific degradation and oxidation of plasma factors and tissue proteins. Recently, the pathobiochemical effects of enzymes of the azurophilic and specific granules of polymorphonuclear granulocytes (elastase, cathepsin G, collagenase, cathepsin B, lysozyme, myeloperoxidase etc.) were investigated in more detail (for review of literature see (1)). Normally, the proteolytic action of the lysosomal proteinases is balanced by proteinase inhibitors present in plasma or other body fluids. During severe inflammation, however, these proteinases may be liberated in amounts exceeding the natural inhibitor levels so that destructive processes can occur either locally or systemically. Evidence could be presented that leukocytic elastase is indeed released during septicemia and other inflammatory processes (1). The correlation observed between the release of this proteinase and the consumption of plasma factors indicates that lysosomal enzymes may significantly contribute to the consumption of plasma factors by unspecific proteolysis. Furtheron, administration of an exogenous inhibitor of lysosomal proteinases in an experimental animal model (endotoxemia) caused a significant reduction of the consumption of plasma factors (2).

Proteinases in Inflammation and Tumor Invasion

Eglin — a proteinase inhibitor which originally has been isolated from the leech Hirudo medicinalis — is a potent inhibitor of the granulocyte proteinases elastase and cathepsin G (3). With regard to the role of these proteinases in inflammatory states like septic shock and trauma evaluation of a therapeutic approach by proteinase inhibition seemed to be desirable. Since eglin is now available in sufficient quantities (Fa. Ciba Geigy/Basel) by genetic engineering (4) experimental animal studies were performed in order to elucidate the inhibitory potency of this inhibitor in sepsis and septic shock.

Methods

Septicemia was induced in pigs by i.v. infusion of E.coli for 2 hours (5). Three different preparations of eglin were intravenously administered for 4 hours: cloned eglin c (batch I or batch II) or eglin b (isolated from the leech). Animals (16.5 - 23 kg) were divided into 7 groups (Tab. 1).

Hematologic, hemodynamic, respiratory, histological and biochemical parameters were measured by routine methods; extravascular lung water (EVLW) was evaluated using the thermo-dye-technique (6). Eglin levels in plasma and urine were estimated by radial immunodiffusion applying specific antibodies (7).

Results

Pharmacokinetic data: Eglin was excreted in urine to 75-95 % within 12 h in animals with normal kidney function. Animals with renal insufficiency due to septicemia showed a significant accumulation of eglin in the circulation indicating the prevailing renal elimination of this inhibitor.

Survival time (Tab. 1): Animals of group II and III died within 7.2 ± 3.0 h and 5.3 ± 1.5 h, respectively. One pig of each of these two groups survived 20 hours. Four animals of group IV survived an experimental period of 30 h despite fever due to traces of endotoxin present in batch I of the cloned eglin c. The mean survival time in group V was 19.1 ± 6 h. In group VI and VII endotoxin-free eglin was used and a remarkable prolongation of survival time was observed in spite of typical hematological, hemodynamic and respiratory disorders due to septicemia.

Group No	n	Bacteria dosage (E.coli cells) in 12 ml phys. NaCl/h for 2 h	Eglin dosage (mg/kg x h) in 50 ml phys.NaCl/h for 4 h	Survival time h
I	10	-	-	30
II	8	3×10^9	-	7.2 ± 3.0
III	9	3×10^{10}	-	5.3 ± 1.5
IV	5	-	3.85 (Eglin c; charge I)	30(4x); 6.5(1x)
V	5	3×10^9	3.85 (Eglin c; charge I)	19.1 ± 9.6
VI	2	3×10^9	4.55 (Eglin b)	30(1x); 4.5(1x)
VII	7	3×10^{10}	3.85 (Eglin c; charge II)	30(5x);15.2(2x)

Table 1: Survival time of pigs in experimental bacteremia as a function of bacteria (E.coli) and inhibitor (Eglin) dosages.

Lung disorders: A significant increase in extravascular lung water was measured in untreated septic animals, whereas during and after eglin-administration only a minor rise in EVLW occured (Fig. 1). Moreover, in contrast to untreated septic pigs eglin-treated animals showed only modest morphological lung alterations (interstitial edema).

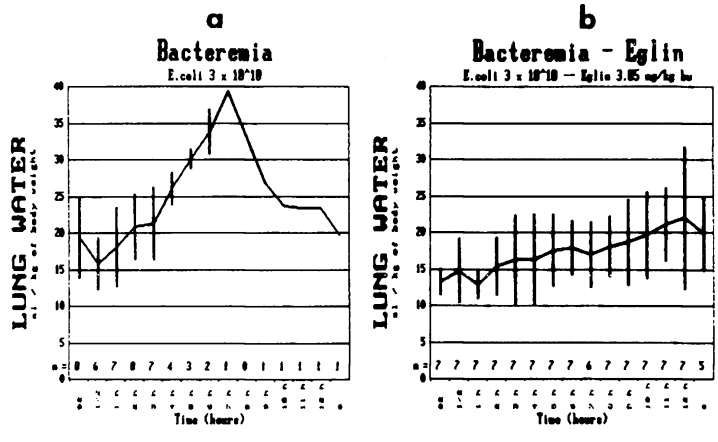

Fig. 1: Mean extravascular lung water levels (EVLW) in septic pigs without (a) and with inhibitor (b) treatment.

Clotting factors: In the eglin-treated groups IV-VII factor XIII and antithrombin III levels decreased transiently (Fig. 2b, 3b), whereas in the untreated bacteremic animals (group II and III) a significant consumption of factor XIII (Fig. 2a) and antithrombin III (Fig. 3a) was measured until death.

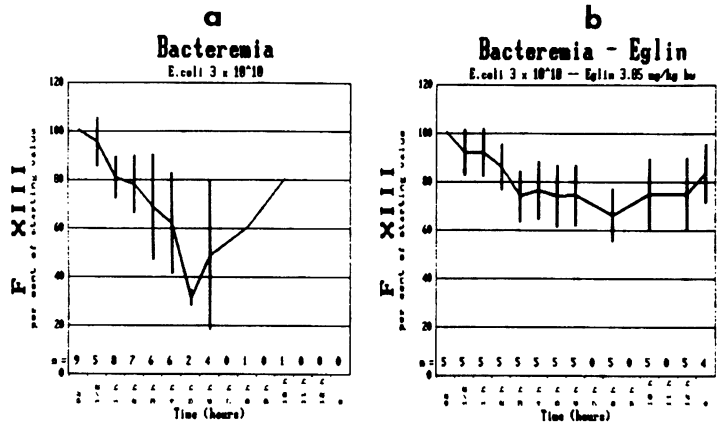

Fig. 2: Mean plasma levels of factor XIII in septic pigs without (a) and with inhibitor (b) treatment.

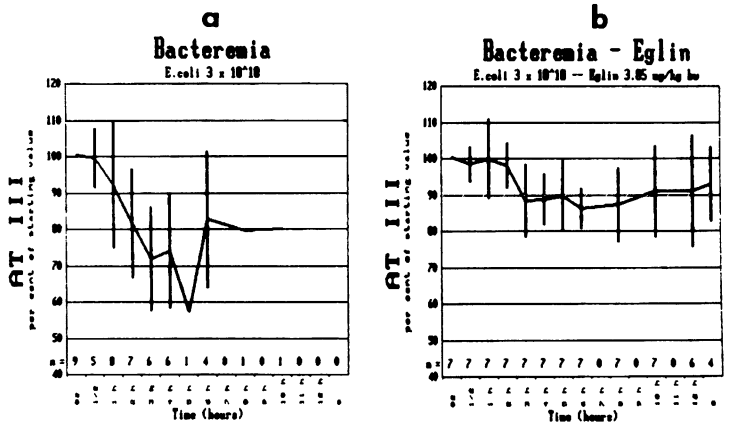

Fig. 3: Mean plasma levels of antithrombin III in septic pigs without (a) and with inhibitor (b) treatment.

Conclusions

Treatment of experimental septicemia in pigs exclusively by eglin revealed a convincing protective effect of this proteinase inhibitor on lung tissue and consumption of plasma proteins probably due to inhibition of unspecific proteolysis. Since, however, some severe organ failures (e.g. renal insufficiency) due to septicemia could not be overcome, a combination of the inhibitor administration with an approved intensive care therapy (e.g. catecholamines, respiratory therapy) is needed for an optimal outcome.

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References

1 Fritz, H., M. Jochum, K.-H. Duswald, H. Dittmer, H. Kortmann, S. Neumann, H. Lang. 1984. Granulocyte proteinases as mediators of unspecific proteolysis in inflammation. A review. In: Selected Topics in Clinical Enzymology, Vol. 2 (eds.: D. M. Goldberg, M. Werner). Walter de Gruyter, Berlin-New York, 306-328.

2 Jochum, M., J. Witte, H. Schiessler, H. K. Selbmann, G. Ruckdeschl, H. Fritz. 1981. Clotting and other plasma factors in experimental endotoxemia: inhibition of degradation by exogenous proteinase inhibitors. Eur. surg. Res. 13, 152-168.

3 Seemüller, U., M. Meier, K. Ohlsson, H. P. Müller, H. Fritz. 1977. Isolation and characterization of a low molecular weight inhibitor (of chymotrypsin and human granuloytic elastase and cathepsin G) from leeches. Hoppe-Seyler's Z. Physiol. Chem. 358, 1105-1117.

4 Rink, H., M. Liersch, P. Sieber, F. Meyer. 1984. A large fragment approach to DNA synthesis: total synthesis of a gene for the protease inhibitor eglin c from the leech Hirudo medicinalis and its expression in E.coli. Nucleic Acids Res. 12, 6369-6387.

5 Welter, H. F., O. Thetter, M. Siebeck, H. Wiesinger, U. Elster, M. Jochum. 1985. Study on the treatment of shock lung with superoxide dismutase (SOD) and C1-inactivator (C1-INA). In: Langenbecks Arch., Suppl. Chir. Forum (ed.: F. Stelzner). Springer-Verlag Berlin-Heidelberg, 63-67.

6 Lewis, F. R., V. B. Elings, J.A. Sturm. 1979. Bedside measurement of lung water. J. Surg. Res. 27, 250-261.

7 Mancini, G., A. O. Carbonara, J. F. Heremans. 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2, 235-254.