Enzyme Activation in Blood-Perfused Artificial Organs

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SYSTEM-SPECIFIC AND LYSOSOMAL (UNSPECIFICALLY DEGRADING) PROTEINASES

The reaction cascades of humoral systems like clotting, fibrinolysis and complement are characterized by individual and highly specific, chiefly enzyme-catalyzed reaction steps. The cascades are triggered again by system-specific proteinases with highly restricted substrate specificity, e.g. clotting by thrombokinases and fibrinolysis by plasminogen activators (cf. Fig. 1). Such cascades triggering proteinases are normally

<table>
<thead>
<tr>
<th>Non-lysosomal Proteinases</th>
<th>Activation of</th>
</tr>
</thead>
<tbody>
<tr>
<td>(high cleavage specificity)</td>
<td>clotting</td>
</tr>
<tr>
<td>Tissue thrombokinases</td>
<td>fibrinolysis</td>
</tr>
<tr>
<td>Plasminogen activators*</td>
<td>complement</td>
</tr>
</tbody>
</table>

* Partly preformed only: synthesis stimulated

FIGURE 1. System-specific, non-lysosomal proteinases present in the organism or its cells in relatively low concentration but their synthesis and/or excretion may be enhanced strongly by either an hormonal or inflammatory stimulus (1). In contrast, lysosomal proteinases are preformed and stored
within the lysosomal granules in fully active form (2). The lysosomal enzymes (cf. Fig. 2) represent an extremely potent

**Lysosomal Proteinases (preformed)**

<table>
<thead>
<tr>
<th>neutral (pH 6-9)</th>
<th>acidic (pH 3-7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Elastases</td>
<td>• Cathepsin B</td>
</tr>
<tr>
<td>• Cathepsin G</td>
<td>• Cathepsins H, L</td>
</tr>
<tr>
<td>° Collagenases</td>
<td>• Cathepsins A, C</td>
</tr>
<tr>
<td>° Kininogenases</td>
<td>• Cathepsin D</td>
</tr>
<tr>
<td>(Kallikreins)</td>
<td>(Leukokininogenase)</td>
</tr>
</tbody>
</table>

**cleavage specificity:** • broad; ° high

**FIGURE 2.** Lysosomal proteinases of neutrophils

hydrolytic capacity which is responsible for the intracellular protein breakdown. In this respect lysosomal proteinases

**Biological Substrates of Neutral Proteinases**

* from Neutrophils

<table>
<thead>
<tr>
<th>Biological Substrates of Neutral Proteinases* from Neutrophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteoglycans</td>
</tr>
<tr>
<td>Elastin</td>
</tr>
<tr>
<td>Collagen I</td>
</tr>
<tr>
<td>Collagen III</td>
</tr>
<tr>
<td>Clotting factors</td>
</tr>
<tr>
<td>Fibrinolysis factors</td>
</tr>
<tr>
<td>Complement factors</td>
</tr>
<tr>
<td>Immunoglobulins</td>
</tr>
<tr>
<td>Transport proteins</td>
</tr>
</tbody>
</table>

* Per day approx. 1.5 g synthezid in men

**FIGURE 3.** Biological substrates of lysosomal proteinases at physiological pH
are able to degrade and thus inactivate either individually or in combination even native proteins and glycoproteins (cf. Fig. 3). Pathobiochemical reactions of both lysosomal and system-specific proteinases are normally prevented by proteinase inhibitors ubiquitously present in the organism (cf. Fig. 4).

<table>
<thead>
<tr>
<th>Abbr.</th>
<th>Inhibitor</th>
<th>M.W. x 1000</th>
<th>Mean conc. mg/100 ml</th>
<th>Mean conc. μmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>α₂M</td>
<td>α₂-Macroglobulin</td>
<td>725</td>
<td>260</td>
<td>3.6</td>
</tr>
<tr>
<td>α₁PI</td>
<td>α₁-Protease inhibitor</td>
<td>50</td>
<td>260</td>
<td>52</td>
</tr>
<tr>
<td>α₁AC</td>
<td>α₁-Antichymotrypsin</td>
<td>70</td>
<td>45</td>
<td>6.4</td>
</tr>
<tr>
<td>β₁CI</td>
<td>β₁-Collagenase inh.</td>
<td>40</td>
<td>1.5</td>
<td>0.4</td>
</tr>
<tr>
<td>III</td>
<td>Inter-α-trypsin inh.</td>
<td>160</td>
<td>45</td>
<td>2.8</td>
</tr>
<tr>
<td>ATIII</td>
<td>Antithrombin III</td>
<td>65</td>
<td>26</td>
<td>4.0</td>
</tr>
<tr>
<td>α₂PI</td>
<td>α₂-Plasmin inhibitor</td>
<td>70</td>
<td>6</td>
<td>0.9</td>
</tr>
<tr>
<td>C1INA</td>
<td>C1-Inactivator</td>
<td>100</td>
<td>24</td>
<td>2.4</td>
</tr>
</tbody>
</table>

FIGURE 4. Proteinase inhibitors in human plasma

MECHANISM OF ACTIVATION AND CONSUMPTION OF PLASMA FACTORS (cf. Fig. 5)

After cell damage, e.g. by endotoxins or trauma, blood system cascades are activated primarily by system-specific proteinases such as thrombokinases or plasminogen activators. Due to the high selectivity of all reaction steps involved, we would like to suggest for this reaction type the term specific activation and, in consequence, for the consumption of factors due to specific activation processes the term specific consumption. In principal, these reactions are characterized by cleavage of one or only a few peptide bonds in a comparatively huge protein molecule resulting in the formation of active enzymes from proenzymes (e.g. thrombin from prothrombin) or accelerators from cofactors (e.g. factor VIIIa from factor VIII). The primary inhibitors present to prevent exceeding activation of the cascades are antithrom-
bin III (AT III) for clotting, $\alpha_2$-plasmin inhibitor ($\alpha_2$PI) for fibrinolysis and C1 inactivator (C1 INA) for complement and kallikrein-kinin system proteinases (cf. Fig. 4 and 5).

FIGURE 5. Activation and consumption reactions: specific activation of blood systems by system-specific proteinases (left part); unspecific degradation of plasma factors by lysosomal neutral proteinases (right part); complex formation with proteinase inhibitors and elimination of the enzyme-inhibitor complexes by phagocytes of the reticulo endothelial system (RES).

Release of lysosomal proteinases from cells besides the mentioned activators may lead to inactivation of blood system factors by unspecific proteolytic degradation and thus to unspecific consumption of plasma factors etc. (cf. Fig. 4). Even proteinase inhibitors like antithrombin III (3) and the C1 inactivator might thus be inactivated, for example, by elastase from polymorphonuclear granulocytes, but also transport proteins (M. Jochum and S. Lander, unpublished observations) and immunoglobulins etc. The primary inhibitors of lysosomal enzymes are $\alpha_1$-proteinase inhibitor ($\alpha_1$PI) (formerly $\alpha_1$-antitrypsin, $\alpha_1$AT), $\alpha_1$-antichymotrypsin ($\alpha_1$AC) and especially $\alpha_2$-macroglobulin ($\alpha_2$M). The latter is able to inhibit nearly all lysosomal proteinases but also sys-
tem-specific proteinases like plasmin, plasma kallikrein and thrombin.

Extensive pathobiochemical reactions by activated system-specific proteinases and/or liberated lysosomal proteinases are normally prevented by the proteinase inhibitors present in excess over the enzymes. Inhibition of the proteinases by the inhibitors occurs relatively rapidly and the stable enzyme-inhibitor complexes thus formed are eliminated within a short time by the cells of the reticulo endothelial system (4).

PATHOBIOCHEMISTRY OF LYSOSONAL ENZYMES

Lysosomal proteinases are of special interest regarding their pathobiochemical potential because of their high amount present in the organism. Very rich in lysosomes are phagocytes like polymorphonuclear granulocytes (neutrophils) and macrophages but also endothelial cells, mast cells and fibroblasts (cf. Fig. 6). Remarkably, the last four cell types are especially

![Diagram of Blood and Tissue Cells with High Numbers of Lysosomes](image)

FIGURE 6. Blood and tissue cells with high numbers of lysosomes

concentrated in lungs, liver and kidneys, i.e. in organs known as primary shock organs; above all, the neutrophils accumulate rapidly in the lungs during an inflammatory response. In view of the fact that approximately 1.3 grams of neutral lysosomal
proteins (primarily elastase and cathepsin G) have to be inhibited and eliminated per day only from disintegrating neutrophils under physiological conditions, it is not surprising that more than 10% of the plasma proteins are represented by proteinase inhibitors. This high inhibitory potential - further inhibitors are present in cytosol and mucous secretions - is a clear additional indication for the pathobiochemical effectiveness of lysosomal proteinases if released extracellularly from their membrane-coated organelles, the lysosomes. On the other hand, a high proteolytic activity in the lysosomes is necessary in order to fulfil their biological functions: (1) degradation of all wasted or potentially dangerous endogenous substances (intracellular catabolism) and (2) degradation of invasive organisms after uptake into the phagosomes of phagocytes (cf. Fig. 7) (5,6,7).

FIGURE 7. Functional significance of lysosomes within the cell. Substances taken up in phagosomes by phagocytosis are degraded subsequently by lysosomal enzymes. (from R. V. Krstić (ed) Ultrastruktur der Säugetierzelle (Springer-Verlag Berlin 1976), modified by the authors of this paper)
SIGNIFICANCE OF PROTEINASE INHIBITORS

During a massive inflammatory process both system-specific and lysosomal proteinases are liberated or accumulate extracellularly in higher concentration leading to a massive consumption or inactivation of the corresponding proteinase inhibitors (8,9,10). Hence, the inhibitory potential of the organism is diminished and thus in turn the effectiveness of the proteinase-induced pathobiochemical processes is enhanced leading to a progressive consumption of factors of the blood systems. Consequences of these processes are severe clinical symptoms including disseminated intravascular coagulation. The justification of this assumption is supported by results of a clinical trial described in the following sections.

LYSOSOMAL ELASTASE AS A DIAGNOSTIC MARKER

FIGURE 8. Complex formation between liberated lysosomal elastase (E) and α₁-proteinase inhibitor (α₁PI) as well as (to a much less extent) α₂-macroglobulin (α₂M). Whereas the E-α₁PI complex is enzymatically inactive, α₂M-bound elastase is still able to hydrolyse low molecular weight (synthetic) substrates.

Neutrophils are stimulated by endotoxins or other inflammatory stimuli thereby releasing an elastase besides other lyso-
somal constituents. Elastase (E) reacts with various susceptible proteins and finally with native $\alpha_1$-proteinase inhibitor ($\alpha_1$PI); the stable enzyme-inhibitor complex (E-$\alpha_1$PI) thus formed (cf. Fig. 8) is eliminated from the circulation with a half-life time of approximately 1 hour. Using a recently developed enzyme-linked immunoadsorbent assay, it is now possible to estimate the concentration of this complex in blood plasma and other body fluids (11,12). $\alpha_1$PI is normally present in body fluids in such a high concentration that liberated elastase is inhibited within a short time, i.e. free active elastase is normally not detectable in plasma samples etc. (A small amount of active elastase may be found; this is due to the presence of elastase in the complex with $\alpha_2$-macroglobulin, where it is still able to catalyse the hydrolysis of synthetic substrates).

LIBERATION OF LYOSOMAL ELASTASE DURING SEPSIS

![Diagram showing patient groups of the prospective clinical trial and number of patients per group.](image)

FIGURE 9. Patient groups of the prospective clinical trial and number of patients per group.

In a prospective clinical trial we studied the course of E-$\alpha_1$PI plasma levels in 3 different groups of patients (cf. Fig. 9) subjected to major abdominal surgery (cf. Fig. 10) (13,14,15):
group A with uncomplicated postoperative course,
group B with postoperative sepsis and recovery,
group C with postoperative sepsis and lethal outcome.

FIGURE 10. Plasma levels of the elastase-$\alpha_1$-proteinase inhibitor complex (E-$\alpha_1$PI) in patients subjected to major abdominal surgery:
A: patients without postoperative infections (n=11)
B: patients who survived postoperative sepsis (n=14)
C: patients who died due to postoperative sepsis (n=16)
The E-$\alpha_1$PI levels are given as mean values for the day before operation, the day after operation, the time before sepsis, at onset of sepsis, and for the course of septicemia. Last determinations were done on day of discharge (D) in group A, on day of recovery (R) in group B, and shortly before death (D) in group C.

In patients without infection signs before operation (group A) normal E-$\alpha_1$PI levels were found (<100 ng/ml). The operative trauma caused a 2 - 3 fold increase in the E-$\alpha_1$PI level which decreased relatively rapidly towards normal levels during recovery (cf. Fig. 10). Postoperative sepsis (cf. Fig. 11), however, was accompanied with highly significantly elevated E-$\alpha_1$PI plasma levels which in turn normalized during recovery (group B, Fig. 10).

Some of the patients (n = 6) of group C showed signs of infection already before operation which is reflected in the preoperatively elevated mean level of E-$\alpha_1$PI (Fig. 10). Post-
Sepsis Criteria

- Defined infection site & pos. bact. culture
- Body temperature > 38.5°C
- Leukocytosis with > 15 000 cells/mm³ or
  Leukocytopenia with < 5 000 cells/mm³
- Platelets < 100 000/mm³ or drop > 30 %
- (Positive blood culture)

FIGURE 11. Criteria used for diagnosis of sepsis

Operatively, a slight decrease of the complex concentration was observed which is probably due to elimination of the infection focus in those patients suffering from an infection already before operation. Onset of sepsis is again accompanied with a highly significant increase of the E-α₁-PI level and this high level was maintained until lethal outcome. Remarkably, in those patients recovering from sepsis (group B) the E-α₁-PI level dropped already significantly during sepsis. This behaviour of the E-α₁-PI plasma level might become a prognostic marker for septic patients. However, the validity of this concept has still to be proven in clinical trials with higher numbers of patients.

CORRELATION BETWEEN ELASTASE RELEASE AND CONSUMPTION OF PLASMA FACTORS

From the clinical trial it is evident that the degree of liberation of a lysosomal marker substance from neutrophils may reflect the severity of the inflammatory response of the organism. Therefore, the search for a possible correlation between the plasma level of E-α₁-PI and factors of the blood systems as postulated above was of major interest. Indeed, a reverse correlation could be established for the following parameters (cf. Fig. 12): antithrombin III, clotting factor XIII and α₂-macro-
globulin. That means, in the given clinical trial high levels of E-α1PI were accompanied with low levels of these factors and vice versa. Obviously, liberation of high amounts of neutrophil elastase is correlated with a high degree of consumption of plasma factors susceptible to elastase cleavage.

### Parameters with High Significance

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sepsis</th>
<th>Survival</th>
<th>Non-survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-α1 PI complex</td>
<td>↑↑</td>
<td>n</td>
<td>↑↑</td>
</tr>
<tr>
<td>Antithrombin III</td>
<td>↓↓</td>
<td>n</td>
<td>↓↓</td>
</tr>
<tr>
<td>Factor XIII</td>
<td>↓↓</td>
<td>n</td>
<td>↓↓</td>
</tr>
<tr>
<td>α2M concent. activity</td>
<td>↓↓</td>
<td>n</td>
<td>↓↓</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>↓↓</td>
<td>n</td>
<td>↑</td>
</tr>
</tbody>
</table>

E: Elastase; α2M: α2-Macroglobulin

FIGURE 12. Correlation between plasma levels of E-α1PI and other plasma factors in the given patients after major abdominal surgery.

The C-reactive protein (CRP), an acute phase reactant, showed a concentration pattern similar to that of E-α1PI; however, CRP proved to be a less specific sepsis indicator compared to E-α1PI (14,15).

**RESUMÉ AND FUTURE PROSPECTS**

The results of the given and ongoing clinical trials indicate that clotting disorders during sepsis are not due exclusively to the activation of blood systems by or via system-specific proteinases (e.g. thrombokinases, plasminogen activator, intrinsic clotting cascade, etc.). Obviously, lysosomal proteinases released from stimulated inflammatory cells are involved to an appreciable degree in the development of consumption coagulopathy or disseminated intravascular coagulation. The underlying mechanism is unspecific proteolytic degradation leading
to inactivation of various blood system factors (3,4,8,16,17).

The given E-α,PI assay is a valuable tool for studying pathological processes in which neutrophils are involved; this concerns besides sepsis and septic shock primarily disorders of the rheumatic type (18) as well as myellogic leukemia (14). In addition, this assay may be used to prove the effectiveness of a proteinase inhibitor therapy (e.g. with aprotinin, the effective ingredient of Trayslo1R) on a broader basis by objectively measurable plasma factors.

REFERENCES


