

PROTEASES

Potential Role in Health and Disease

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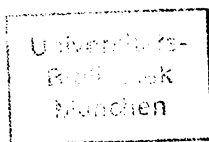
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PROTEOLYTIC ACTIVITY IN PATIENTS WITH HYPERCATABOLIC RENAL FAILURE

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INTRODUCTION

Despite several advances in dialysis and medical therapy, the mortality rate for patients with acute renal failure (ARF) remains distressingly high. When ARF is associated with major surgery or trauma, the mortality rate is about 50 to 70 %. Such patients are often hypercatabolic as a result of sepsis, hemorrhage, or open-draining wounds. They may be wasted or malnourished from underlying illnesses. Losses of glucose, amino acids and proteins during hemodialysis or peritoneal dialysis contribute to wasting.

Total parenteral nutrition with amino acids and hypertonic glucose has been reported to: stabilize or reduce serum levels of urea nitrogen, potassium and phosphorous improve wound healing; enhance survival from ARF and possibly increase the rate of recovery of renal function¹. However, nitrogen balance remains negative in all patients² suggesting that decreased protein synthesis is not the sole cause of loss of body nitrogen. Several studies have shown that proteinases participate in protein catabolism of patients with hypercatabolic ARF³⁻⁶. This report summarizes data concerning the potential role of proteases in enhanced protein breakdown in patients with acute and chronic renal failure.

MATERIAL AND METHODS

Proteolytic activity in plasma, dialysate and urine fractions was measured, as previously described, using as substrates phosphorylase kinase (from rabbit skeletal muscle), azocasein or a particular fraction of hepatocytes³⁻⁷. Polyacrylamide gel electro-

phoresis in the presence of sodium dodecylsulfate was carried out according to Weber and Osborn⁸. The inhibitory activity of alpha₁-protease inhibitor (alpha₁-antitrypsin) was determined with a commercial test system (Boehringer, Mannheim, FRG). Plasma concentrations of alpha₂-macroglobulin (α_2 M) and alpha₁-protease inhibitor (α_1 PI) were evaluated by a radial immunodiffusion technique with standardized immunodiffusion plates (Behringwerke, Marburg, FRG). Quantitative estimation of the plasma levels of the elastase-alpha₁-protease inhibitor (E- α_1 PI) complex was carried out with a highly sensitive enzyme-linked immunoassay⁹. We report here the results obtained in: 12 healthy subjects aged from 22 - 39 years (seven males, five females), 12 chronically uremic patients aged from 22 - 66 years undergoing regular dialysis therapy (RDT) for 41.3 ± 11.6 month (nine males, three females) and 10 patients with posttraumatic ARF aged from 19 - 45 years. Every 4th day venous blood was collected immediately before and after dialysis. Furthermore, dialysate and urine fractions were collected weekly on ice in the presence of 0.01 % azide.

RESULTS

Ultrafiltrated plasma fractions, obtained from healthy subjects and RDT patients and prepared by ultrafiltration with an Amicon XM 50 filter, had no effect on degradation of phosphorylase kinase. However, the ultrafiltrated plasma fractions, obtained from patients with posttraumatic ARF, were proteolytic. Figure 1 shows the digestion of the three subunits of phosphorylase kinase after incubation with ultrafiltrated plasma for 1 min as well as 10 and 24 hours. One minute after incubation, the typical subunit structure of this enzyme with the three polypeptide chains: alpha (MW 135,000), beta (MW 120,000) and gamma (MW 42,000) was observed. Ten hours later, the alpha subunit was partially degraded, whereas the gamma subunit was completely digested. After 24 hours the whole enzyme was destroyed. Phosphorylase kinase was also digested by plasma ultrafiltrates of the other nine patients with posttraumatic ARF within 5 - 36 hour incubation period at 37 °C.

Free proteolytic activity in the plasma may be a consequence of the low alpha₂-macroglobulin values, whereas the concentrations of alpha₁-protease inhibitor (alpha₁-antitrypsin) were markedly enhanced. Figure 2 shows the trypsin binding capacity of plasma taken from patients with posttraumatic ARF and from RDT patients. The levels of proteolytic activity (after the addition of increasing amounts of trypsin) are significantly higher in patients with posttraumatic ARF compared with RDT patients indicating a lower trypsin binding capacity of the plasma in both groups of patients compared with healthy subjects.

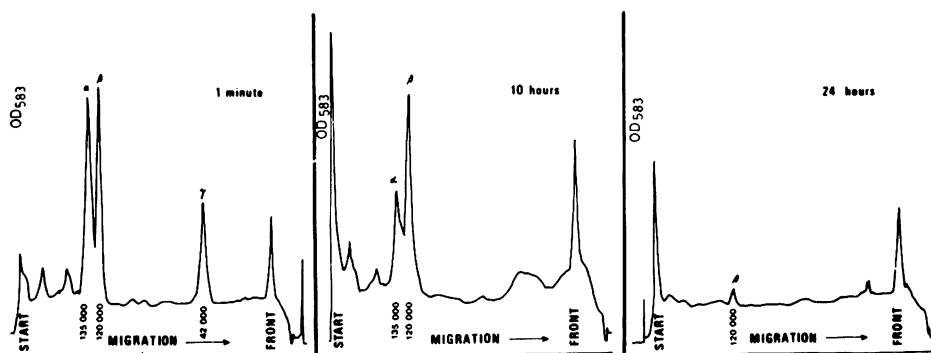


Fig. 1: Effect of an ultrafiltrate of plasma from patient H.H. on phosphorylase kinase. 0.1 ml ultrafiltrate (2.1 mg/ml), obtained by ultrafiltration of plasma through an Amicon XM 50 filter, was added to 0.1 ml phosphorylase kinase (1.5 mg/ml). Samples (1 min, 10 and 24 hr after incubation) were subjected to polyacrylamide (5 %) gel electrophoresis in the presence of sodium dodecylsulfate⁸.

The effect of hemodialysis therapy on plasma proteinase activity, leukocyte counts, plasma elastase- α_1 -protease inhibitor (E- α_1 PI) complex as well as α_1 PI activity and plasma concentration is shown in table 1. The proteolytic activity of the plasma fractions was measured using azocasein as a substrate. Before hemodialysis we found a significant increase in RDT patients (+ 244 %; $p < 0.01$) compared with plasma samples of healthy subjects (0.052 ± 0.004 U/mg protein). The highest plasma proteolytic activity was observed in patients with posttraumatic ARF (0.255 ± 0.044 U/mg). During hemodialysis therapy, however, there was a permanent decrease of plasma proteinase activity (0.127 ± 0.019 vs. 0.037 ± 0.007 U/mg; -71 %; $p = 0.001$). Leukocyte counts decreased 10 min (21.1 %; n.s.) and 30 min (41.1 %; $p < 0.001$) after initiation of hemodialysis therapy. We observed a maximal increase of the plasma E- α_1 PI complexes after three hours (+ 409 %; $p < 0.001$). In contrast, plasma α_1 PI activity and α_1 PI concentration were unchanged during hemodialysis therapy.

A parallel digestion of the subunits alpha, beta and gamma of phosphorylase kinase was observed after incubation of urine fractions of patients with posttraumatic ARF for 12 hours. However, when approx. 100-times concentrated dialysates (pore size 10,000 dalton) were incubated with phosphorylase kinase the alpha polypeptide chain of the enzyme was completely degraded within 1 hour. After 4 hr of incubation the gamma subunit was also totally destroyed, whilst approx. 2/3 of the beta chain was digested.

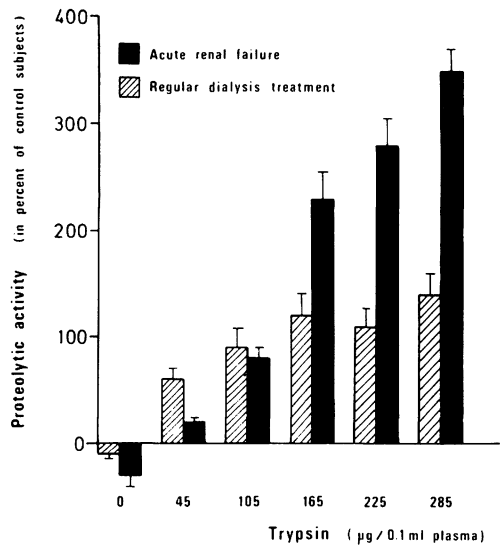


Fig. 2: Proteolytic inhibitory capacity in the plasma of RDT patients and patients with posttraumatic ARF expressed in percent of healthy subjects. 0.1 ml of plasma from experimental subjects were incubated for 60 min at 37 °C with increasing amounts of bovine trypsin. Proteolytic activity was measured using azo-casein as a substrate⁷. Means \pm SEM from five experiments.

Table 1

Effect of hemodialysis therapy on plasma proteinase activity, leukocyte counts, plasma elastase- α_1 -proteinase inhibitor (E- α_1 PI) complex as well as α_1 PI activity and plasma concentration

	0	10	30	60	120	180 minutes
Proteinase activity (U/mg protein)	0.127 \pm 0.019	0.103 \pm 0.014	0.095 \pm 0.014	0.087 \pm 0.013	0.059 \pm 0.010 ^b	0.037 \pm 0.007 ^c
Leukocytes (cells/mm ³)	7,133 \pm 582	5,625 \pm 696	4,200 \pm 251 ^c	6,658 \pm 441	5,950 \pm 607	7,192 \pm 824
E- α_1 PI (ng/ml)	188 \pm 20	196 \pm 25	326 \pm 56 ^a	373 \pm 32 ^c	602 \pm 84 ^c	769 \pm 128 ^c
α_1 PI (U/ml)	1.98 \pm 0.11	1.89 \pm 0.11	2.07 \pm 0.10	2.06 \pm 0.09	2.01 \pm 0.09	2.08 \pm 0.11
α_1 PI (mg/dl)	254 \pm 14	257 \pm 13	268 \pm 13	258 \pm 12	260 \pm 11	271 \pm 13

Mean values \pm SEM from 12 experiments before and during hemodialysis therapy
(^a_p < 0.05; ^b_p < 0.01; ^c_p < 0.001)

DISCUSSION

The object of our study was to investigate the role of proteases on the hypercatabolic state associated with ARF.

Figure 1 shows the proteolytic digestion of phosphorylase kinase by ultrafiltrated plasma from a hypercatabolic patient with posttraumatic ARF. Comparable data were obtained with plasma ultrafiltrates from all patients with posttraumatic ARF.

Plasma ultrafiltrates from normal subjects and patients with ARF after drug overdosage (barbiturate, flurazepam, carbromal, different analgesics, aminoglycosides or cytostatics) did not affect the subunit structure of phosphorylase kinase within 24 hours incubation⁴. Furthermore, plasma ultrafiltrate of patients with posttraumatic ARF also digests hepatocyte membrane fractions, whereas control ultrafiltrate only demonstrates very faint proteolytic degradation within 24 hours of incubation at 37 °C⁶.

Normally, digestion of protein in the plasma is effectively limited by inhibitors of proteases. Alpha₁-antitrypsin (alpha₁-protease inhibitor) and alpha₂-macroglobulin accounted for more than 90 % of the total protease inhibiting capacity of plasma¹⁰. It has been shown that plasma alpha₁-antitrypsin level increases in patients with ARF particularly after multiple traumatic injuries. In contrast to alpha₁-antitrypsin, the values of plasma alpha₂-macroglobulin were markedly decreased⁴. Free proteolytic activity in the plasma may be a consequence of the relative low alpha₂-macroglobulin values. However, these inhibitors were measured by immunological techniques and their concentration shows no relationship to the biological activity of these inhibitors. Trypsin binding capacity was significantly lower in RDT patients and dramatically reduced in patients with posttraumatic ARF compared to healthy controls.

We have previously shown that proteolytic activity of the administered plasma protein solutions are lower than proteolytic activity of plasma fractions of healthy controls⁴. Thus we can conclude that no proteolytic activity was infused into our patients with ARF. However, alpha₂-macroglobulin was not detectible in the available plasma protein solutions in contrast to relative low concentrations of alpha₁-antitrypsin⁴. Free proteolytic activity in the plasma of a patient with posttraumatic ARF was observed due to protease-antiprotease imbalance. After an initial determination of 80 mg/dl the alpha₂-macroglobulin values were too low to be detected. Addition of purified alpha₂-macroglobulin to the ultrafiltrates resulted in complete inhibition of phosphorylase kinase digestion in vitro⁵. According to our studies we favour the application of alpha₂-macroglobulin or fresh frozen plasma to hypercatabolic

patients. Balldin et al. have demonstrated that the availability of alpha-macroglobulin is of vital importance for protection against proteinases¹¹. Elastase¹² and trypsin¹³ are bound by alpha-macroglobulin. Increased catabolism of alpha-macroglobulins after intravenous infusion of trypsin-alpha₁-antitrypsin complexes has been observed in dogs. More than half of the trypsin molecules were taken up by alpha-macroglobulins during the first hour. Subsequent to saturation of alpha-macroglobulins dogs became shocked¹¹.

Proteolytic degradation of the subunits alpha, beta and gamma of purified phosphorylase kinase was obtained by urine fractions of patients with posttraumatic ARF⁶, whereas urine fractions of patients with nephrotic syndrome led to a prevalent digestion of the alpha polypeptide chain¹⁴.

When dialysates of RDT patients were concentrated approx. 100-times we also observed rapid digestion of phosphorylase kinase⁴. From these experiments we may assume that proteinase may also be involved in the protein catabolism of RDT patients. Furthermore, it may be possible to remove in part proteolytic enzymes during dialysis treatment.

Hemodialysis therapy has been reported to be a catabolic event. It has been shown that glucose in the dialysate¹⁵ as well as continuous amino acid infusion¹⁶ are ineffective to prevent this catabolic state. The pathogenesis of hemodialysis induced protein catabolism remains unclear. One possibility could be the release of granulocyte proteinases after starting hemodialysis therapy. Craddock et al. have demonstrated hemodialysis-induced leukopenia and pulmonary vascular leukostasis resulting from complement activation by dialyzer cellophane membranes¹⁷.

Neutrophil granulocytes contain a broad variety of agents that are involved in the defence and digestion of invading microorganisms¹⁸. These include elastase^{18,19}, cathepsin G, proteinase-3, cathepsin B, cathepsin D and collagenase²⁰⁻²⁵. Lysosomal proteinases are not restricted to the intracellular compartment. They are readily released extracellularly during cell death, phagocytosis, exposure to antigen antibody complexes, complement components and toxic substances such as endotoxins^{26,27}. Under pathological conditions massive protease release may occur. This results in tissue injury^{28,29} and degradation of plasma proteins, if the activities of the controlling proteinase inhibitors in plasma and tissues are insufficient.

Recently, Aasen et al.³⁰ observed in an experimental study (lethal endotoxin shock in canines) a clear relation of the initial drop of leukocytes (probably combined with degranulation of these cells) to the appearance of the leukocyte elastase- α_1 -proteinase in-

hibitor complex in plasma. Shortly thereafter, the well-known disturbances of the blood systems arose indicating that consumption of their components might be due, at least in part, to the action of liberated leukocytic proteinases.

Granulocyte elastase may be released during hemodialysis treatment probably due to the contact of blood cells with the blood lines and the dialyzer membrane (cuprophane) system. This contact may result in a "frustrated phagocytosis" and a subsequent proteinase release. However, leukopenia during hemodialysis therapy does not parallel the increase of E- α_1 PI. Pulmonary vascular leukostasis resulting from complement activation by dialyzer cellophane membranes may be involved in this process¹⁷. Plasma α_1 PI activity and α_1 PI concentration were unchanged during hemodialysis therapy (Table 1).

Alpha₁-protease inhibitor is a dominating protease inhibitor in plasma. It forms complexes with virtually all serine proteinases^{31,32} and some bacterial proteinases. A variety of oxidants including myeloperoxidase produced by neutrophils as well as cigarette smoke are capable of inactivating alpha₁-protease inhibitor³³⁻³⁵.

With decreased alpha₁-antiproteinase concentration inhibition of bacterial and leukocyte proteinases in the bronchial secretion may be insufficient or absent resulting in alveolar damage and emphysema³⁶. In idiopathic pulmonary fibrosis progression of the disease is assumed due to a proteinase/antiproteinase imbalance. Active collagenase has been observed in the lavage fluid of the lower respiratory tract³⁷.

Our results offer the possibility that the hemodialysis-induced release of elastase may enhance the risk for development of destructive lung disease in long-term RDT patients.

Proteolytic activity of the plasma fractions using azocasein as a substrate was higher in RDT patients (+ 244 %; $p < 0.01$) compared with healthy controls (0.052 ± 0.004 U/mg protein). However, proteinase activity decreased permanently during hemodialysis therapy indicating an activation of the plasma inhibitory capacity. In agreement with this hypothesis we observed a further decrease of plasma proteinase activity during hemodialysis therapy after the addition of pork pancrease elastase to the plasma samples obtained³⁸.

SUMMARY

1) Proteolytic enzymes exist in ultrafiltrated plasma, concentrated dialysates and urine fractions of patients with posttraumatic renal failure. Differences in digestion pattern of phosphory-

lase kinase suggest the existence of different proteases in patients with hypercatabolic renal failure.

2) Trypsin binding capacity is reduced in RDT patients and markedly lower in patients with posttraumatic ARF.

3) Protein catabolism is inhibited in vitro by α_2 -macroglobulin. From our in vitro studies we favour the application of fresh frozen plasma instead of the available plasma protein solutions to hypercatabolic patients.

4) Hemodialysis may enhance proteinase inhibitory capacity of the plasma.

5) Hemodialysis therapy induces the increase of plasma E-X₁ PI. The continuous release of granulocyte elastase during hemodialysis therapy may enhance the risk for the development of destructive lung disease.

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