Factor XIII and Fibronectin

New clinical and biological approaches

R. Egbring and H.-G. Klingemann
Editors

First International Conference
on Factor XIII and Fibronectin
July 16–17, 1982
Philipps-University, Department of Haematology,
Marburg/West-Germany

Die Medizinische Verlagsgesellschaft mbH, Marburg/Lahn 1983

Contents

Fibronectin session

[1]Abteilung für Klinische Chemie und Klinische Biochemie in der [2]Chirurgischen Klinik Innenstadt der Universität München,
[3]Medizinische Klinik III im Klinikum Großhadern der Universität München, West-Germany

Plasma levels of neutrophil elastase-α_1-proteinase inhibitor complexes and factor XIII (including subunits A & S) in septicemia and leucemia

M. Jochum[1], *K.-H. Duswald*[2], *E. Hiller*[3], and *H. Fritz*[1]

Introduction

During the inflammatory response various or local tissue cells are activated thereby releasing internal, mostly lysosomal enzymes. Due to the liberation of these enzymes depletion of clotting factors in septicemia or acute leucemia may occur via two major routes (fig. 1):

- *System-specific* proteinases, such as thrombokinases and plasminogen activators, trigger the activation of the clotting,

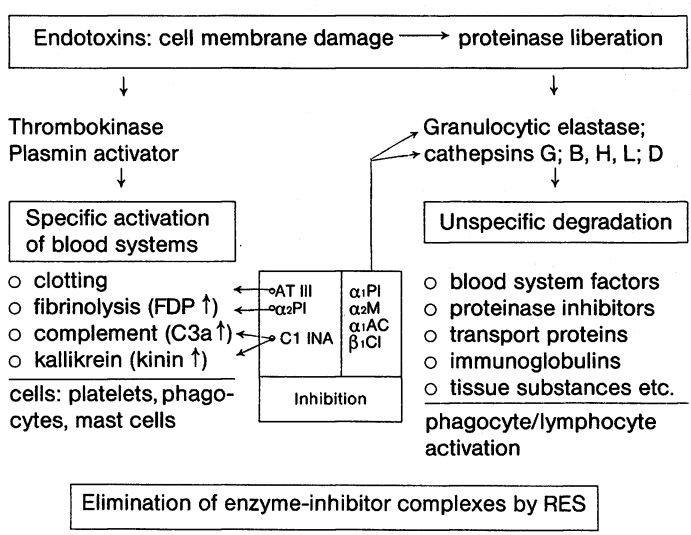

Fig. 1. Consumption of plasma factors during inflammation. For details see text. Abbreviations: Antithrombin III (AT III), α_2-plasmin inhibitor (α_2PI), C1 activator (C1 INA), α_1-proteinase inhibitor (α_1PI, formerly α_1-antitrypsin), α_2-macroglobulin (α_2M), α_1-antichymotrypsin (α_1AC), β_1-collagenase inhibitor (β_1CI), fibrin(-ogen) degradation products (FDP), complement factor (C3a), reticuloendothelial system (RES).

fibrinolysis, and complement cascades (summarized as »blood systems«) by *substrate-specific* proteolysis of proenzymes or cofactors. Due to the subsequent inhibition and rapid elimination of the enzyme/inhibitor complexes via the reticuloendothelial system (RES) not only the proteinases but also their specific inhibitors (e. g. antithrombin III, α_2-antiplasmin, C1-inactivator) are consumed. For a long time the given sequence of reactions was assumed to be exclusiveley responsible for the development of disseminated intravascular coagulation.

- Results obtained recently[1, 2, 3, 4] indicate that an additional reaction pathway contributes considerably to the consumption of plasma factors during severe inflammations. This implies inactivation of plasma proteins by *substrate-unspecific* proteolysis due to liberated lysosomal proteinases such as elastase and cathepsin G from polymorphonuclear granulocytes (neutrophils).

Especially elastase may destroy blood proteins before inhibition occurs by the plasma proteinase inhibitors, α_1-proteinase inhibitor (α_1PI, formerly α_1-antitrypsin) and α_2-macroglobulin. On account of these potent inhibitors, direct measurement of the granulocytic proteinase activities in plasma is not possible. However, increased levels of the elastase-α_1 PI complex (E-α_1PI) would be an indirect but clear indication of elastase liberation. Indeed Egbring and coworkers have demonstrated such complexes by means of one- and two-dimensional Laurell electrophoresis in plasma of patients suffering from acute leucemia or septicemia[1].

In the studies performed in our laboratory we were especially interested to see whether a relationship exists between the levels of E-α_1PI complex and the severity of postoperative infections after major abdominal surgery, or clotting factor depletions in acute leucemia.

To achieve this purpose, besides other factors, the levels of factor XIII (measured by a modified F XIII-Schnelltest from Behringwerke Marburg and by rocket-immunoelectrophoresis) and antithrombin III (determined by the coatest antithrombin from Deutsche Kabi, München) were continously monitored, because both clotting factors are known to be easily degraded by neutrophil elastase in vitro[5, 6].

Quantitative estimation of the plasma levels of the elastase-α_1-proteinase inhibitor complex was carried out with a *highly sensitive enzyme-linked immunoassay*. This assay has been

developed recently by Neumann and coworkers[7] and was adapted to clinical conditions in our laboratory.

Briefly, the E-α_1PI complex in the plasma sample is bound to surface-fixed antibodies directed against neutrophil elastase. After washing with buffer, a second alkaline phosphatase-labelled antibody directed against α_1PI is fixed to the complex. Under suitable conditions, the activity of the bound alkaline phosphatase

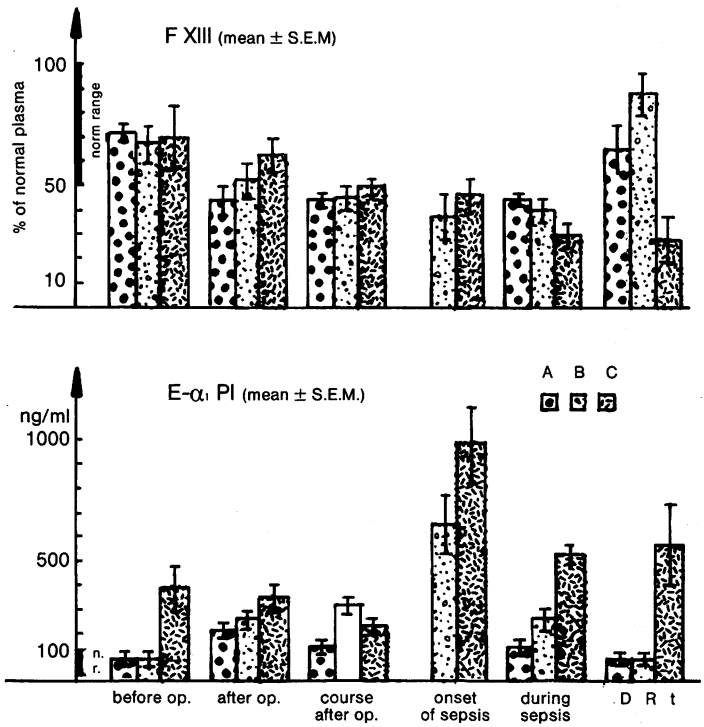

Fig. 2. Plasma levels of elastase-α_1-proteinase inhibitor complex (E-α_1PI) and fibrin stabilizing activity of factor XIII (F XIII) in patients subjected to major abdominal surgery.
A = patients (n = 11) being without postoperative infection
B = patients (n = 14) surviving postoperative septicemia
C = patients (n = 16) dying as a result of septicemia.
The E-α_1PI levels are given as mean values (± SEM) for the day before operation, the day after operation as well as for the postoperative phase before sepsis, at onset of sepsis and during septicemia. Last determinations were done on day of discharge (D) for group A, on day of recovery (R) for group B, and before death (+) for group C.
nr = normal range

towards p-nitrophenylphosphate is proportional to the concentration of the E-α_1PI complex in the sample.

Results and Discussion

a) Levels of elastase-α_1PI complex and factor XIII in patients after major surgery

In the prospective clinical study[8] more than 120 patients were included. Thirty of them fulfilled defined septic criteria during the postoperative course. Of these patients fourteen survived the infection (group B), whereas sixteen died as a direct result of septicemia (group C). Eleven patients, being without infection after abdominal surgery, served as controls (group A).

With the enzyme-linked immunoassay normal levels of complexed elastase were found between 40 and 150 ng/ml in 153 healthy individuals (mean value: 86.5 ± 25.5 ng/ml).

As shown in fig. 2, in patients without preoperative infection (group A and B), the operative trauma was followed by an increase of the E-α_1PI level up to 3-fold of the normal value. Patients suffering from preoperative infections showed already clearly elevated preoperative complexed elastase levels. Immediately after surgery a slight decrease was observed, probably due to elimination of the infection focus.

At the beginning of septicemia a highly significant increase of the complexed elastase could be detected: up to 6-fold in group B and up to 10-fold in group C. Peak levels were found above 2.500 ng/ml in both groups. The E-α_1PI levels of septic patients who recovered showed a clear tendency towards normal values. In patients with persisting septicemia, high levels of complexed elastase were measured until death.

In comparison to the E-α_1PI changes throughout the postoperative phase, the factor XIII activity showed an inverse pattern (fig. 2). A decrease in F XIII activity far below the normal value could be observed in all three groups during the early postoperative course. At onset of sepsis no statistically significant difference, either within the infected groups or between these groups and the control, could be demonstrated. However, throughout the course of septicemia, the factor XIII activity in plasma of patients who did not survive decreased further up to 28% of the standard mean value. Last determinations showed a clear tendency towards normal in patients of the control group and of the group overcoming the infection. In

group C patients the mean value was still below 30% of the norm concomitantly to the highly elevated levels of E-α_1PI.

These patients also had very low concentrations of both: subunit A comprising the active enzyme, and subunit S representing the carrier protein (data not shown). In control patients, however, only the F XIII activity and subunit A exhibited low values during the early postoperative phase, whereas subunit S was diminished unsignificantly.

Interestingly, during clotting, only subunit A is consumed but not subunit S. Elastase, however, is able to degrade both subunits to a similar degree as demonstrated clearly by Egbring and coworkers[1, 5]. These data and the results presented in our clinical trial suggest that in the patients suffering from septicemia, unspecific proteolytic degradation by neutrophil elastase and/or other lysosomal proteinases is involved to a significant degree in the depletion of F XIII.

The changes of the most important plasma factors after major surgery are summarized in fig. 3: Simultaneously to the rise of E-α_1PI complex a marked decrease of antithrombin III, factor XIII and α_2-macroglobulin as well as an increase of the acute phase reactant C-reactive protein was measured during sepsis. These plasma factors all normalized in patients who overcame the

Parameter	Sepsis	Survival	Non-survival
E-α_1 PI complex	↑↑	n	↑↑
Antithrombin III	↓↓	n	↓↓
Factor XIII	↓↓	n	↓↓
α_2 M concent.	↓↓	n	↓↓
α_2 M activity	↓↓	n-↓	↓↓
C-reactive protein	↑↑	n	↑
E: Elastase: α_2M: α_2-Macroglobulin			

Fig. 3. Correlation of the increase of elastase-α_1-proteinase inhibitor complex (E-α_1PI) and the decrease of antithrombin III (AT III), factor XIII (F XIII) and α_2-macroglobulin (α_2M) as well as the increase of the C-reactive protein (CRP) in plasma of patients suffering from septicemia after major abdominal surgery.
n = normal value

Patients	Diagnosis	Leukocytes (x $10^3/\mu l$)	E-α_1PI (ng/ml)	F XIII activity (% of the norm)	AT III activity (% of the norm)
Kh	AML	3.5	27	87.5	87
Re	AML	4.0	66	62.5	98
Ec	AML	10.6	96	37.5	66
Schö	AML	163.0	170	50.0	70
Hi	AML	4.1	640	62.5	83
Bo	AML	17.9	840	50.0	88
Ha	AML	140.0	1140	50.0	98
We	APL	1.6	1320	87.5	104
Si	APL	52.0	1600	n. d.	n. d.
Me	CML	7.8	132	120	109
Bo	CML	174.0	336	75.0	109
Ri	CML	381.0	750	75.0	110
Ay	CML	76.0	1000	120	107
He	ALL	32.0	30	37.5	85
Sa	ALL	35.0	64	25.0	70
Ri	ALL	69.5	68	25.0	100
Schi	ALL	36.0	76	37.5	105

Table 1. Leucocyte counts and plasma levels of elastase-α_1-proteinase inhibitor complex (E-α_1PI), factor XIII, and antithrombin III in patients with acute myelogenous leucemia (AML), acute promyelocytic leucemia (APL), chronic myelogenous leucemia with blastic transformation (CML), or acute lymphatic leucemia (ALL), respectively. For details see text.

infection disease, whereas they showed pathological values in patients with fatal outcome. We take this as a clear indication that especially neutrophil proteinases contribute significantly to the inflammatory response of the organism.

b) Levels of elastase-α_1PI complex and factor XIII in acute leucemia

In our preliminary study of elastase release throughout leucemia[9], seventeen patients suffering from various kinds of leucemia were included (table I).

Prior to chemotherapy, six out of nine patients with acute myelogenous leucemia (AML) had moderate to high levels of E-α_1PI (170–1.440 ng/ml), the highest levels being found in the two patients with acute promyelocytic leucemia (APL; up to 1.600 ng/ml). This type of acute leucemia is frequently associated with disseminated intravascular coagulation. Elevated E-α_1PI levels

(130–1.000 ng/ml) were also measured in the plasma of four patients suffering from chronic myelogenous leucemia with blastic transformation (CML). In contrast, none of the four patients with acute lymphatic leucemia (ALL) had increased levels of E-α_1PI.

Although the fibrin stabilizing activity of F XIII was frequently reduced in AML patients no clear correlation could be found between the absolute amount of elevated levels of E-α_1PI and the decrease in F XIII activity.

Surprisingly, the ALL patients showed extremely low F XIII activity although their E-α_1PI levels were in the normal range. In contrast, CML patients with blastic transformation and increased E-α_1PI levels showed normal or even elevated F XIII activity. Additionally, in contrast to Egbring and coworkers[1], no clear correlation between elevation of E-α_1PI complex and decrease of subunit S could be demonstrated (data not shown).

The AT III activity was in the normal range, except for three patients. Their low AT III level, however, was also not correlated to a high E-α_1PI concentration.

These preliminary data suggest that the mechanism of F XIII depletion in acute leucemia seems to be not directly dependent on elastase liberation, and should be therefore, different from that postulated for septicemia. Unfortunately, there is no explanation for this discrepancy at the moment.

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 51, München.

We are very grateful to Drs. S. Neumann and H. Lang, Biochemical Research Institute of E. Merck, Darmstadt, for providing us with the E-α_1PI enzyme-linked immunoassay.

The excellent technical assistance of Mrs. U. Hof and Mrs. C. Seidl is greatly appreciated.

References

[1]*Egbring, R., W. Schmidt, G. Fuchs, K. Havemann:* Demonstration of granulocytic proteases in plasma of patients with acute leucemia and septicemia with coagulation defects. Blood *49:* 219–231 (1977). – [2]*Egbring, E., M. Gramse, N. Heimburger, K. Havemann:* In vivo effects of human granulocyte neutral proteinases on blood coagulation in green monkeys (cercopithecus aetiops). 6th Int. Congr. Thromb.

Diath. Haemorrh. *38:* 222 (1977). – [3]*Aasen, A. O., K. Ohlsson:* Release of granulocyte elastase in lethal canine endotoxin shock. Hoppe-Seyler's Z. Physiol. Chem. *359:* 683–690 (1978). – [4]*Jochum, M., J. Witte, H. Schiessler, H. K. Selbmann, G. Ruckdeschl, H. Fritz:* Clotting and other plasma factors in experimental endotoxemia. Inhibition of degradation by exogenous proteinase inhibitors. Eur. surg. Res. *13:* 152–168 (1981). – [5]*Schmidt, W., R. Egbring, K. Havemann:* Effect of elastase-like neutral protease from human granulocytes on isolated clotting factors. Thromb. Res. *6:* 315–326 (1974). – [6]*Jochum, M., S. Lander, N. Heimburger, H. Fritz:* Effect of human granulocytic elastase on isolated human antithrombin III. Hoppe-Seyler's Z. Physiol. Chem. *362:* 103–112 (1981). – [7]*Neumann, S., N. Hennrich, G. Gunzer, H. Lang:* Enzyme-linked immunoassay for human granulocyte elastase α_1-proteinase inhibitor complex. In: D. M. Goldberg and M. Werner (Eds.): Progress in Clinical Enzymology-II 1982 (in press). – [8]*Duswald, K. H., M. Jochum, H. Fritz:* Released granulocytic elastase: an indicator of pathobiochemical alterations in septicemia after abdominal surgery. Submitted to Surgery. (1982). – [9]*Jochum, M., K. H. Duswald, E. Hiller, H. Fritz:* Plasma levels of human granulocytic elastase-α_1-proteinase inhibitor complex (E-α_1PI) in patients with septicemia and acute leucemia. In: D. M. Goldberg, M. Werner, (Eds.): Progress in Clinical Enzymology-II 1982 (in press).

Discussion

Rasche:

You told us that elastase inhibitor complexes are elevated in patients with acute leucemia. I would be interested in what stage of acute leucemia has blood samples been taken. I am especially interested if you have some data of those patients, who had a drug induced bone marrow depression.

Jochum:

Unfortunately I cannot tell you something about the stage of acute leucemia, because we have received the samples from Dr. Hiller from Munich and there was not enough time to get some information about the disease. But we also had samples after chemotherapy in acute myelocytic leucemia and the starting values of the complexes were very high and they came down to normal during chemotherapy and also the factor XIII activity came up to normal value at the end of the chemotherapy.

Rasche:

I think the question seems very important to me, because what is with the elastase-inhibitor complexes during the phase of hypoplastic bone marrow?

Jochum:

We have in the meantime only one patient after chemotherapy, so I cannot answer your question sufficiently.

Discussant:

Several of the last speakers have alluded to possible connection between raised levels of elastase and lower levels of factor XIII. We all know that DIC and septicemia are very complex disease states and I do not really feel convinced that elastase is the only factor responsible for the factor XIII decline.

Egbring:

We did not say that it is the only reaction. I have, in the first slide, showed that three mechanisms are responsible for degradation of patients with septicemia, including DIC, possibly including hyperfibrinolysis. And another point is that factor XIII may be degraded by bacterial proteinases. That is a hypothesis, but we will attempt to prove it.