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H. PEETERS

*Lipid and Protein Department
Institute for Medical Biology
Brussels, Belgium*



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**CLOTTING AND OTHER PLASMA
FACTORS IN SEPTICEMIA:
INHIBITION OF DEGRADATION BY
AN EXOGENOUS PROTEASE
INHIBITOR FROM SOYBEANS
(TYPE: BOWMAN-BIRK)**

M. JOCHUM, J. WITTE, H. SCHIESSLER and H. FRITZ

*Department of Clinical Chemistry and Biochemistry, Surgical Clinic
University of Munich, Federal Republic of Germany*

ABSTRACT

In the course of Gram-negative septicemia, septic shock, or experimental endotoxemia granulocytic proteinases such as elastase and cathepsin G are suggested to be released by stimulation with endotoxin. In vitro proteolysis of several plasma factors by granulocytic elastase is almost completely prevented by prior inhibition of elastase with a proteinase inhibitor from soybeans (Bowman-Birk inhibitor, BBI). In experimental endotoxemia in dogs, degradation of various plasma factors could also be significantly diminished by prior systemic application of this elastase-cathepsin G inhibitor (BBI). These results indicate that the consumption reaction observed during endotoxemia and septicemia is a biphasic event. Besides activation of the clotting and fibrinolytic systems by system-specific proteinases such as thrombokinase or plasminogen activator, the decrease of the levels of plasma factors is caused also to an appreciable degree by unspecific proteolytic degradation due to granulocytic proteinases.

KEYWORDS

Septicemia; endotoxemia; endotoxin; disseminated intravascular coagulation (DIC); unspecific proteolysis; granulocytic proteinases; proteinase inhibitor.

INTRODUCTION

Human polymorphonuclear (PMN) leukocytes contain at least three neutral proteinases (elastase, cathepsin G, collagenase) which are released by stimulation with endotoxin. This could be particularly demonstrated for granulocytic elastase in the course of acute myeloid leukemia and septicemia (Egbring and co-workers, 1977) as well as in canine endotoxin shock. (Aasen and Ohlsson, 1978). The activities of clotting and other plasma factors either in their isolated form or in plasma are strongly reduced by digestion with elastase or cathepsin G (Johnson and co-workers, 1976; Schmidt and co-workers, 1974).

Even in vivo the reduction of such factors could be due to degradation by granulocytic proteinases under certain pathological conditions (Egbring and Havemann, 1978).

The activity of the granulocytic proteinases is regulated in vivo by plasma inhibitors such as α_1 -antitrypsin, α_2 -macroglobulin and α_1 -antichymotrypsin and by antileukoprotease in mucous secretions (for references see Aasen and Ohlsson, 1978). The in vitro interaction of granulocytic elastase and cathepsin G with inhibitors of animal, plant and microbial origin is well investigated (Schiessler and co-workers, 1977). Only recently, inhibition of granulocytic proteinases could be demonstrated indirectly after application of an elastase-cathepsin G inhibitor from soybeans (BBI; MW \sim 8000) in the course of experimental Gram-negative septicemia in dogs (Schiessler and co-workers, 1978). As infusion of BBI prevented the rapid fall in activity of the fibrin-stabilizing factor F XIII, the authors suggested that this decrease might be also caused by nonspecific proteolysis rather than by the action of thrombin throughout severe disseminated intravascular coagulation (DIC) which often complicates the pathophysiological events in septicemia. The purpose of this investigation was to show that the decrease in clotting and other plasma factors during septicemia, septic shock, or experimental endotoxemia is not only due to DIC (Müller-Berghaus and Lasch, 1975) but also to an appreciable degree to unspecific proteolysis by granulocytic proteinases.

RESULTS

In vitro, purified human granulocytic elastase degraded isolated clotting factors (F VII, F Xa, F XIII) and the transport protein transferrin rapidly within 5-30 minutes even at a molar ratio of 2 (elastase) to 100 (plasma protein). This could be demonstrated by PAGElectrophoresis and immunoelectrophoresis according to Laurell. Additionally, the major inhibitor of clotting factors antithrombin III is also rapidly degraded by elastase thereby losing its biological activity. This was shown by crossed immunoelectrophoresis as well as with an activity assay using the chromogenic substrate S-2238 from Deutsche Kabi, München (for detailed information: M. Jochum, H. Lander, and H. Fritz, manuscript in preparation). Proteolytic degradation and loss of activity was almost completely inhibited by prior incubation of elastase with BBI.

The plasma levels of antithrombin III and α_2 -macroglobulin as well as those of various clotting (fibrinogen, F XIII), complement (C3, C4) and other plasma factors (transferrin, prealbumin, inter- α -trypsin inhibitor) were found to be significantly decreased in 18 patients suffering from hyperdynamic septic shock (experimental details are given in the habilitation script of Witte, 1979 and are submitted for publication). A similar statistically significant reduction of the concentrations and activities of several plasma proteins (prothrombin, antithrombin III, factor F XIII, plasminogen, α_2 -plasmin inhibitor, and complement factor C3) was observed in experimental endotoxemia in dogs (M. Jochum, J. Witte, H. Schiessler, G. Ruckdeschel, and H. Fritz, manuscript in preparation). In this model, the decrease of the plasma proteins was considerable diminished by prior intravenous injection of the elastase-cathepsin G inhibitor BBI (Fig. 1).

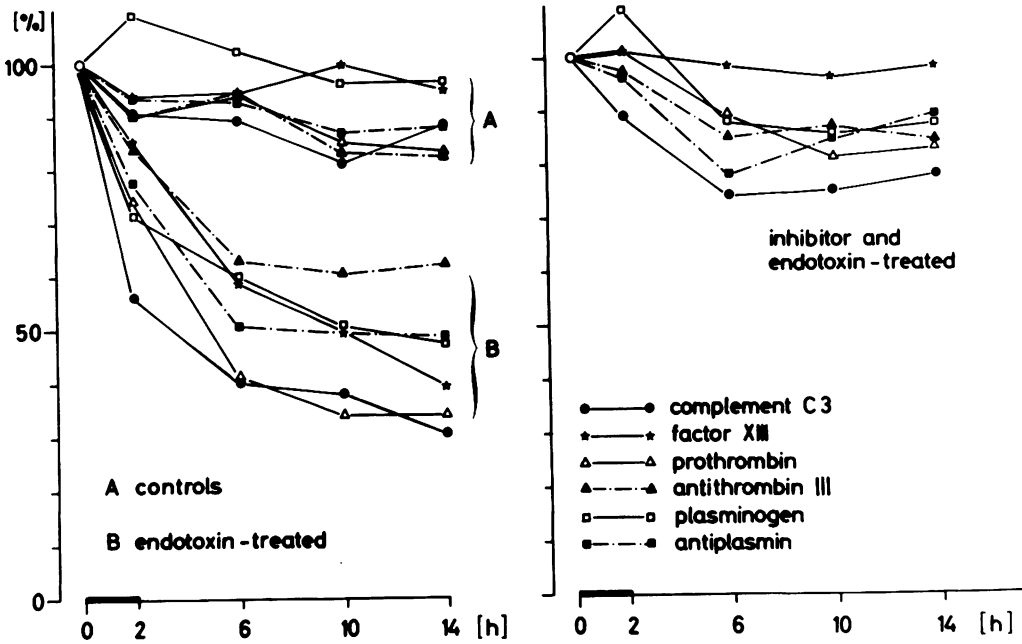


Fig. 1 Plasma levels of several plasma proteins during experimental endotoxemia without (a) and with (b) Bowman-Birk inhibitor medication. At the ordinate the percentage of the starting plasma level of each factor is given. At the abscissa the experimental period and the endotoxin infusion period (thick line) are indicated. For further details see Witte, 1979.

DISCUSSION

Marked consumption or turnover of plasma proteinase inhibitors during septic shock reflect extensive activation of the clotting, fibrinolytic, and complement systems (summarized as "blood systems") as well as the liberation of considerable amounts of proteinases from various body cells, especially from granulocytes (for references see Fritz, 1980). System-specific proteinases such as plasminogen activator and thrombokinase activate the blood systems by specific proteolytic cleavages, i.e. by proenzyme→enzyme conversion. leading to severe consumption of plasma proteins. The release of granulocytic proteinases causes furthermore considerable unspecific degradation of the plasma factors as could be demonstrated indirectly by systemic application of an elastase-cathepsin G inhibitor (BBI) in the course of experimental endotoxemia. However, quantitation of the degree of consumption due to unspecific proteolysis is not feasible yet. The results of our experimental and clinical studies indicate clearly that in generalized inflammatory processes like septicemia or septic shock the

regulatory inhibitor potential of the organism may be overstressed. Systemic application of suitable proteinase inhibitors may prevent at least the unspecific degradation of plasma factors and therefore contribute to maintain the physiological balance. As medication with BBI or other proteinase inhibitors is not possible in humans until toxicological, pharmacological and clinical trials have been successfully elaborated, substitution with antithrombin III and α_2 -macroglobulin is the method of choice at present.

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