Activation of platelets and coagulation during haemodialysis
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Activation of coagulation during treatment with haemodialysis

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ABSTRACT

Generation of factor XII, thrombin antithrombin complexes, prothrombin fragment 1+2 and thrombus precursor protein has been monitored in 16 subjects during haemodialysis.

Immediately after starting treatment, contact of blood with the negatively charged surfaces of the polyacrylnitril membrane AN-69 resulted in a 9 - 45% decrease in factor XII activity. Peak concentrations for thrombin antithrombin complexes (50 to 120 µg/L) were observed 30 min after the start of haemodialysis. Establishment of thrombus precursor protein concentrations yielded steadily increasing results without any tendency to decrease during treatment.

Determination of thrombin antithrombin complexes is considered to establish the most sensitive short-term reacting parameter indicating activation of coagulation. A steady generation of fibrin and fibrinogen-fibrin complexes during treatment with haemodialysis is indicated by increasing results for thrombus precursor protein. In order to prevent clotting during haemodialysis, an additional supplementation of anticoagulant is needed.
INTRODUCTION

Extracorporeal circulation of blood disturbs the haemostatic balance in subjects treated with haemodialysis (HD) [1, 2]. In particular, the degree of hypercoagulability is strongly influenced by surface characteristics of the dialyzer membrane and flow conditions within the circuit [3, 4]. Evaluation of conditions concerning the haemocompatibility of membranes, of flow rates and application of anticoagulants is essential in minimizing damage due to blood cell activation [5, 6].

AN-69 membranes are characterized as highly biocompatible. However, contact of blood with negatively charged surfaces like the polyacrylnitril membrane AN-69 may result in specific binding of coagulation factor XII [7]. Because activation of factor XII is induced by mediation of the prekallikrein-kininogen complex, platelet activation may stimulate this conversion by creating a procoagulant surface [8]. As a result, thrombin is generated which converts fibrinogen into fibrin. Fibrin monomers polymerize or are incorporated in complexes with intact fibrinogen, degradation products of fibrinogen and fibrin. Immediate precursors of insoluble fibrin are indicated as thrombus precursor protein (TpP) [9]. The conversion of prothrombin into active thrombin is a key major event in the final stage of the coagulation cascade. Therefore the concentration of prothrombin fragment 1+2 (F1+2) will yield valuable information with regard to the actual amount of thrombin that has been formed.

Until now, time-dependent curves for registration of the accumulation of activation markers in the course of HD have only been published for the initial stage of treatment with AN-69 membranes [5].

The aim of the current study has been to reveal more information with respect to kinetics of factor XII, accumulation of thrombin-antithrombin III complexes (TAT), prothrombin fragment 1+2 and thrombus precursor protein polymers during the complete period of treatment with HD.

SUBJECTS AND METHODS

Subjects
Sixteen subjects between the ages of 20 and 80 years, having undergone HD three times a week, participated in the study. Treatment with HD had been indicated because of renal failure due to pyelonephritis, nephrolithiasis, glomerulosclerosis, membrane proliferative glomerulonephritis, IgA nephropathy or renal cysts. In the remaining cases, nephropathy was of unknown origin. Subjects were treated three times a week in periods ranging from 2 to 12 years.

Subjects were included in the study after informed consent had been received. Criteria for exclusion were application of salicylates or other therapy which might affect platelet function. After venipuncture, blood samples were anticoagulated with sodium citrate (0.129 mol, 1/10). Platelet-poor plasma was prepared by centrifugation of blood at 2500g for 10 min at 4°C. Small aliquots of plasma were stored in plastic tubes at -70°C until analysis. Blood samples were collected from the arterial line before dialysis (t=0), after first passage (t=1 min) and subsequently after 5 (t=5), 30 (t=30) and 150 (t=150) min.
Dialysis protocol
The polyacrylnitril membrane AN-69 (Hospal, Uden, The Netherlands) and the biBAG dialysis system (Fresenius, Bad Homburg, Germany) were applied. Blood flow rates ranged between 200 and 250 ml/min and ultrafiltration flow rates between 300 and 1000 ml/min according to the individual need of the patient. Dialysate contained sodium at a concentration of 138 mmol/L, potassium at 2 mmol/L, calcium at 1.75 mmol/L, magnesium at 1.5 mmol/L, chloride at 107 mmol/L, glucose at 5.5 mmol/L and acetate at 38 mmol/L. The dialysis sessions lasted 3 - 4 h, depending on individual needs and efficacy of the treatment.

A continuous intravenous infusion (100 - 800 U h⁻¹) of low molecular weight heparin (Fragmin, Kabi, Stockholm, Sweden) was applied for anticoagulation purposes during HD. Individual amounts were estimated after consideration of the dosage needed at the previous treatment with HD. At the start of dialysis, a priming dosage depending on individual body weight and amounting to 4000 - 6000 units low molecular weight heparin (LMWH) was administered. In one subject a bolus dosage of 2000 units LMWH was supplemented after 2 h. Before starting HD, the extracorporeal system was rinsed with approximately 500 ml of saline.

Factor XII
Factor XII activity was determined by performing a modified activated partial thromboplastin time (APTT) with silica activation. Samples were diluted and added to plasma deficient in factor XII. Correction of the prolonged clotting time of the deficient plasma was proportional to the activity percentage of factor XII in the patient’s plasma. Calibration was performed with application of CALplasma as supplied by the manufacturer (Instrumentation Laboratory, Milano, Italy).

Thrombin antithrombin complexes
The concentration of thrombin-antithrombin III complexes (TAT) was determined by means of a sandwich-type enzyme-linked immunosorbent assay (Enzygnost® TAT micro, Dade Behring, Marburg, Germany).

Prothrombin fragment 1+2
The concentration of prothrombin fragment 1+2 (F1+2) was determined by means of a sandwich-type enzyme-linked immunosorbent assay (Enzygnost® F1+2 micro, Dade Behring, Marburg, Germany).

Thrombus precursor protein
The concentration of thrombus precursor protein polymers (TpP) was determined by application of an enzyme-linked immunoassay (ABS TpP® Assay, Kordia, Leiden, The Netherlands).

Fibrinogen
Fibrinogen concentration was established in accordance with the Clauss method (10) by adding an excess of thrombin to diluted plasma in order to convert fibrinogen to fibrin (Instrumentation Laboratory, Milano, Italy).
Statistics
Statistical evaluation of data was performed by applying multivariate analysis (ANOVA) and Student’s t-test for paired results (SPSS software). Differences were considered to be statistically significant if $p<0.05$.

Figure 1. Results of factor XII activity (%) established at several stages in 16 subjects before haemodialysis (1), t=1 (2), t=5 (3), t=30 (4) and t=150 min (5) after starting HD. The lower level of the reference range is indicated by the horizontal, dashed line.

Figure 2. Results indicating activation of coagulation established at several stages in 16 subjects before HD (1), t=1 (2), t=5 (3), t=30 (4) and t=150 min (5) after starting HD. A. TAT (μg/L). B. F1+2 (nMol/L). C. TpP (mg/L). The upper level of the reference range is indicated by the horizontal, dashed line. The subject with application of an additional heparin dosage is marked with a - - - - line.
RESULTS

The current study is designed to register kinetics along with intraindividual variations due to activation of coagulation during treatment with HD.

**Factor XII**

As demonstrated in Figure 1, decreased levels of factor XII concentrations beyond the lower level of the reference range were observed in four subjects. In these cases, levels remained at the lower level of the reference range during treatment with HD. In the complete subjects’ group, a definite decrease occurred in the first minute, amounting to a mean value of 28% (range 9 - 45%). Afterwards, only slight further increases were detected. Finally, towards the end of treatment, results equalled the initial values established before starting HD.

**TAT and prothrombin F1+2**

Thrombin generation was estimated following the generation of TAT (Figure 2A) and F1+2 (Figure 2B). Determination of TAT concentrations yielded increased results after only 5 min (9.5±1.8 µg/L, mean±SEM), whereas obvious peak values from 50 to 120 µg/L were established 30 min after the start of dialysis. A level of moderately increased values was maintained until the end of dialysis (29.6±3.0 µg/L, mean±SEM).

F1+2 concentrations were demonstrated to be constant during the initial stage (0 - 5 min) of treatment with dialysis; when compared with the reference range, increased results were established in 15 out of 16 participating subjects. Peak values (9.6±0.7 nMol/L, mean ± SEM) were reached at t=30 min. Although peak values decreased slightly, values still remained obviously increased after terminating HD.

**Figure 3.** Results for fibrinogen concentration (g/L) established at several stages in 16 subjects before HD (1), t=1 (2), t=5 (3), t=30 (4) and t=150 min (5) after starting HD. The upper level of the reference range is indicated by the horizontal, dashed line.
**TpP**

TpP concentrations within the reference range were demonstrated as early as during the initial stage in 12 out of 16 subjects (Figure 2C). At $t=30$ min in 11 out of 16 subjects, increased results were observed compared with the reference range. At $t=150$ min, a still steadily ongoing increase was observed.

Increased fibrinogen concentrations were initially established for 11 subjects (Figure 3). At the final stage of treatment with HD, values beyond the reference range were observed for 14 subjects.

**DISCUSSION**

For subjects undergoing HD, no standardized procedure for anticoagulation with heparin or other anticoagulants is available to prevent clot formation. Extended monitoring of TAT and D-dimer concentrations can be applied for adjusting the heparin dosage per subject in combination with a biocompatible membrane [11].

Increased TAT concentrations are indicative of activation of coagulation with AN-69 membranes [12]. AN-69 membrane biocompatibility has already been evaluated in healthy volunteers but only for an initial test period of 27 min [5]. When applying AN-69 membranes, only low and comparable levels of activation of coagulation parameters have been reported [5]. In contrast, results from the present study demonstrate obvious deviations due to HD, whereas wide ranges of interindividual variations occur. Concentrations of various coagulation products demonstrated obvious fluctuations in subsequent stages of treatment.

Immediately after starting treatment with HD, contact of blood with the negatively charged artificial surface of the dialysis membrane resulted in an unequivocal decrease of factor XII activity.

Factor XII is activated when bound to negatively charged surfaces and may subsequently cleave prekallikrein to yield kallikrein. Activated factor XII can also activate factor XI, which catalyses the activation of factor IX and hence that of the intrinsic pathway of coagulation. Kallikrein and activated factor XII can activate plasminogen in the intrinsic pathway of fibrinolysis [14].

Blood samples collected from the arterial line during dialysis reflected a state of hypercoagulation in the dialysis circuit.

Distinct peak values for TAT concentrations are observed as little as 30 min after the start of dialysis. A subsequent decrease in TAT concentrations can be explained by a reduction of contact activation due to protein coating within the dialyzer membrane [15].

Owing to the short half-life time (<15 min) it is concluded that TAT is a sensitive and very short-term reacting parameter indicating activation within the coagulation pathway [13]. Simultaneously, only a steadily proceeding increase is demonstrated for F1+2 concentrations with no decline towards lower results at the final stage of dialysis. Before starting HD, F1+2 values still exceeded the upper level of the reference range. Results from our investigations are not in agreement with the conclusion of another study [5] that F1+2 would be an earlier marker of thrombin generation than TAT complexes. Apparently, the effects of essentially different mechanisms have been detected. The theoretical base of these observations has not yet been elucidated.
Fibrin generation, fibrinous deposits and subsequent degradation may result from complex biological processes, including ischaemia, increased platelet aggregability and inflammatory responses [16, 17].

Steadily increasing results for TpP concentrations are indicative of proceeding generation and degradation from fibrin and fibrinogen after thrombin generation due to membrane activation of the coagulation cascade. It has been hypothesized that increased fibrinogen concentrations in the case of HD, which are shown in Figure 3 would contribute to disturbances of haemostatic balance in favour of hypercoagulability. However, a statistically significant relationship between fibrinogen and TAT concentrations cannot be established for the results of this study.

After application of alternative membranes, for instance F500S, F60 and TRICEA, initial contact activation of coagulation appearing from increases in TAT concentration has been observed to some extent (own observation). A reduction of initial coagulation activation is of essential clinical importance. As a consequence of severe clotting, the efficacy of treatment is reduced, resulting in longer duration of dialysis. In the case of clotting more blood will remain within the dialyzer membrane.

Readjustment of the dosage of heparin is recommended if TAT concentrations exceed levels of 20 mg/L immediately after the start of HD [18]. In this respect, elevated concentrations of fibrinogen, as demonstrated in our study, are considered to be an increased risk factor for activation of coagulation [18]. In borderline cases, the appearance of clump formation in the afferent line of the dialyzer system, a reduction of hypercoaguability and a risk of clot formation by increasing the heparin dosage should be demonstrated in practice in further investigations. The same consideration holds when generation of fibrin during treatment with HD is indicated by increased TpP results.

More effective supplementation of anticoagulants is recommended to prevent activation of coagulation. In our opinion the various parameters just mentioned should be applied conscientiously as a means of further optimizing treatment with anticoagulant during HD.
REFERENCES


