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Increased thrombin generation in patients with chronic renal failure

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Abstract The plasma concentration of prothrombin fragment 1+2 (F1+2) is considered a very sensitive parameter for specific detection of latent hypercoagulability. To evaluate the degree of hypercoagulation associated with chronic uremia, we measured F1+2 by ELISA in the plasma of 51 patients with severe or end-stage chronic renal failure (35 males, 16 females, aged 22–81 years): 24 on dietary treatment, 15 on combined dietary and once a week hemodialysis, and 12 on regular maintenance hemodialysis; 33 healthy subjects served as a control group. Plasma F1+2 showed a significant elevation in the group on dietary treatment; it was further increased in the group on once a week hemodialysis, and even more markedly increased in the group on maintenance hemodialysis. In patients on dietary treatment a positive correlation was found between plasma F1+2 and serum creatinine. In patients on maintenance hemodialysis, no increase in the F1+2 plasma level was found during the course of a single hemodialysis session. Low molecular weight heparin, administered to 7 patients on dietary treatment, caused a marked drop in the F1+2 plasma level, providing evidence that the elevation in F1+2 indicates an accelerated *in vivo* thrombin generation rather than impaired renal catabolism. The enhanced coagulation activation appears to be related to the reduction of residual renal function, i.e., to the severity of renal failure, and may contribute to the increased risk of vascular events in uremic patients.

Key words Prothrombin fragment 1+2 · Hypercoagulation · Calcium nadroparin · Chronic uremia

Introduction

Elevated plasma concentrations of fibrinogen, factor VII, factor VIII procoagulant, von Willebrand factor, and factor XIII [1–6], as well as low plasma concentrations of anti-thrombin III, protein C anticoagulant activity, and free protein S [5–10], have been found in patients with chronic renal failure, suggesting the existence of hypercoagulation. In a previous investigation we documented elevated plasma levels of thrombin-antithrombin complex, fibrinopeptide A, and D-dimer in chronic uremia: the subset of patients on maintenance hemodialysis (MHD) showed significantly higher level of thrombin-antithrombin complex and D-dimer than the subset of patients on conservative dietary treatment (DT), providing evidence that uremics on MHD have a higher degree of hypercoagulation, possibly related to the risk of thromboembolic complications [11].

The measurement of prothrombin fragment 1+2 (F1+2) is considered to quantify factor Xa-induced prothrombin activation to thrombin, thereby providing a very sensitive tool for specific detection of latent hypercoagulability [12]. Recently an ELISA has been developed for the quantification of F1+2 in plasma, using a specific polyclonal antibody that discriminates between native prothrombin and F1+2 by recognizing the carboxy-terminal amino acids of F1+2 [13, 14].

To further assess the degree of hypercoagulation associated with chronic uremia, we have measured F1+2 in the plasma of three groups of chronic uremic patients: on conservative DT, on once-a-week hemodialysis (OHD), and on MHD. In some patients on DT we also evaluated the effect of a low molecular weight heparin on the plasma concentration of F1+2. We have found evidence of increased *in vivo* prothrombin conversion into thrombin; this may be of concern for the development of cardiovascular complications, which represent the leading cause of death in patients with chronic uremia [15].

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Materials and methods

Patients

Fifty-one adult patients with severe or end-stage chronic renal failure were studied (Table 1). Twenty-four patients were on conservative treatment with a very low-protein (0.3 g/kg per day), low-phosphorus diet supplemented with essential amino acids and ketoanalogues for 6 months at least. Patients with urinary protein excretion greater than 3 g/24 h were excluded. Fifteen patients were on a combined dietary and OHD schedule [16]. The other 12 patients were on regular MHD, undergoing 4 h of hemodialysis three times a week. In all cases, hemodialysis was performed via an arteriovenous fistula on the forearm; a hollow-fiber dialyzer was used, and sodium heparin was administered in the inlet line at a priming dose of 500–1,000 IU, followed by a continuous infusion of 500–1,000 IU/h.

Ten patients (5 on DT, 1 on OHD, 4 on MHD) had evidence of ischemic heart disease (5 stable angina, 3 unstable angina, 2 previous myocardial infarction). Two patients (1 on DT and 1 on MHD) had chronic atrial fibrillation. One patient on DT had a previous cerebral transient ischemic attack. Two patients (1 on DT and 1 on MHD) had peripheral obstructive arterial disease (intermittent claudication). Two patients (1 on OHD and 1 on MHD) had had a previous deep venous thrombosis of the lower limbs. Arteriovenous thrombotic occlusion had occurred in 6 patients (4 on OHD and 2 on MHD).

During the study, none of the patients received drugs affecting the hemostatic system, with the exception of the heparin administered during the hemodialysis sessions. Subjects with recent (less than 1 month) or acute thrombosis, bleeding, fever, infections, diabetes mellitus, liver diseases, or malignancy were excluded. No individual was tested in the 2 weeks following a surgical procedure or invasive investigations. Thirty-three healthy subjects, matched for age and sex with the patients, served as a control group.

Collection and processing of blood samples

Blood was drawn by clean venipuncture without stasis in plastic syringes containing 3.8% sodium citrate solution 1:10 (v/v); samples were discarded if venipuncture was judged imperfect. Blood samples from hemodialysis patients, including both MHD and OHD subjects, were collected by puncture of the arteriovenous fistula (inlet line) immediately before the start of hemodialysis. In order to evaluate the change, if any, induced by the hemodialysis procedure, blood samples from MHD patients for F1 + 2 analysis were also obtained from the outlet line at the start of the hemodialysis session, and from the inlet and the outlet line at the end of the hemodialysis session. The blood samples were spun at 2,000 × g for 20 min at 4 °C, and the citrated plasma obtained was stored at –20 °C and tested within 3 months.

Table 1 Main clinical and laboratory data of the uremic patients (C_{Cr} creatinine clearance, DT dietary treatment, OHD once-a-week hemodialysis, MHD maintenance hemodialysis)

Patient group (sex)	Age (years) mean ± SD (range)	Creatinine (mg/dl) mean ± SD (range)	C_{Cr} (ml/min) mean ± SD (range)	Hemodialysis age (months) mean ± SD (range)
DT (16 M, 8 F)	44 ± 13 (21–66)	6.6 ± 2.4 (2–10)	11.6 ± 12 (2.8–56)	
OHD (11 M, 4 F)	51 ± 14 (29–73)	12.9 ± 1.7 ^a (9.5–16)	2.4 ± 0.7 ^a (1.3–3.7)	10.8 ± 10 (2–37)
MHD (9 M, 3 F)	58 ± 12 (24–81)			14.3 ± 8 (9–37)

^a Values determined prior to the hemodialysis session

Hemostasis measurements

F1 + 2 was measured by ELISA (Enzygnost F1 + 2, Behringwerke, Scoppito, Italy) [13]. The results were expressed in nanomoles per liter. The intra-assay coefficient of variation was 6.1% for normal values and 6.9% for values above 5 nmol/l. The interassay coefficient of variation was 8.2% for normal values and 9.7% for high values. The normal range was defined as the mean ± 2 SD of controls. The following hemostatic measurements were also performed: prothrombin time, activated partial thromboplastin time, fibrinogen, and platelet count.

Low molecular weight heparin administration

Seven consecutive patients on DT received an intravenous injection of a single dose of 0.4 ml low molecular weight heparin (calcium nadroparin, Seleparina, Italfarmaco, Milan, vials 0.4 ml = 4,100 IU aXa). Blood for F1 + 2 analysis was collected immediately before, 60 and 120 min after the nadroparin injection. Informed consent was obtained for each patient according to the principles of the Helsinki Declaration.

Statistical analysis

Results were expressed as mean ± SD. Differences between groups were analyzed for significance using analysis of variance (one-way); Student's *t*-test was used for paired data. Correlation coefficients were calculated for the different variables using the Pearson's correlation test. Differences were considered significant when $P < 0.05$.

Results

Overall, plasma levels of F1 + 2 were higher in the population of 51 uremic patients than in healthy controls (2.24 ± 1.04 vs. 1.04 ± 0.42 nmol/l, $P < 0.001$). The three groups of uremic patients were considered separately. As shown in Table 2, the patients on DT had higher F1 + 2 plasma levels than healthy controls ($P < 0.05$). The patients on OHD had higher F1 + 2 plasma concentrations than uremic patients on DT ($P < 0.05$) (Table 2). A further increase in the F1 + 2 plasma level was observed in the patients on MHD compared with patients on OHD ($P < 0.05$) (Table 2). The difference in F1 + 2 levels between DT and MHD patients was even more marked ($P < 0.0001$).

A positive correlation was found between plasma F1 + 2 and serum creatinine in patients on DT (correlation coef-

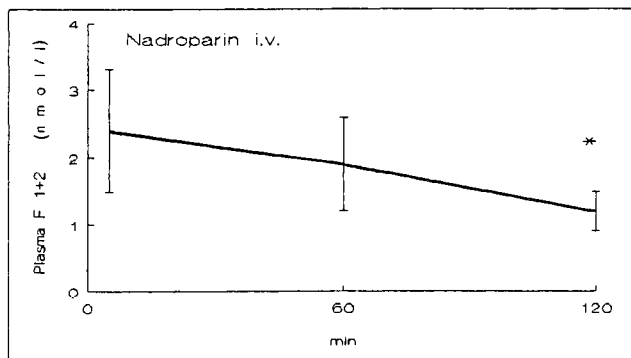


Fig. 1 Plasma levels of prothrombin fragment 1+2 (F1+2) before, 60 min and 120 min after intravenous (*i.v.*) injection of calcium nadroparin (4,100 U aXa). * Student's *t*-test $P < 0.05$

Table 2 Plasma levels prothrombin fragment 1+2 (nmol/l) in healthy controls, uremics on DT, on OHD, and on MHD

Healthy controls (<i>n</i> = 33)	DT uremics (<i>n</i> = 24)	OHD uremics (<i>n</i> = 15)	MHD uremics (<i>n</i> = 12)
1.04 ± 0.42	1.70 ± 0.56	2.25 ± 0.89	3.28 ± 1.20

(Mean ± SD)

ficient 0.63, $P < 0.05$). Calcium nadroparin, administered intravenously to 7 patients on DT, caused a significant decrease in the plasma F1+2 level at 120 min (from 2.40 ± 0.91 to 1.20 ± 0.30 nmol/l, $P < 0.05$), as shown in Fig. 1.

In MHD patients no change in the F1+2 plasma level was found during a single hemodialysis session: the mean F1+2 at the end of hemodialysis (samples obtained from the inlet line) was 3.20 ± 1.62 nmol/l (NS vs. predialysis values, Table 2). Furthermore, samples obtained from the inlet and the outlet line at the start as well as the end of the hemodialysis session did not show any difference in F1+2 levels. There was no significant difference in the F1+2 plasma level between uremic patients with a history of thrombosis and those with no previous thrombotic event.

Discussion

Elevated concentrations of F1+2 have been found in a number of conditions associated with elevated thromboembolic risk, namely insulin-dependent diabetes mellitus, nephrotic syndrome, Crohn's disease, and familial deficiency of antithrombin III and protein C [17]. Moreover, it has been demonstrated that F1+2 concentrations are reduced in patients on oral anticoagulant treatment or sodium heparin infusion [14, 18, 19]. To date, there have been very few investigations of plasma F1+2 levels in patients with chronic uremia [20–22]. To our knowledge, plasma F1+2 levels have not been assessed in patients with renal failure of differing severity and treated with different therapeutic regimens.

Our study shows that plasma F1+2 is significantly elevated in uremic patients on DT; it is further increased in uremic patients on OHD and more markedly elevated in uremic patients on MHD. Significant differences in mean plasma concentrations of F1+2 were found between the three groups of uremic patients; the lower the residual renal function, the higher the F1+2 plasma level. It is well established that there is a general increase in the mean plasma F1+2 level with advancing age [23]. The various subsets of uremics studied and the group of healthy controls were well matched for age, therefore any age-related influence on F1+2 level could be reasonably excluded.

It is not known, at present, whether excess generated F1+2 is metabolized or excreted through the kidney. The increase in F1+2 we observed in chronic uremia might indicate an increased *in vivo* cleavage of the prothrombin molecule by factor Xa (i.e., hypercoagulation) or might merely be due to impaired renal catabolism of this 271-amino acid peptide. To address this question, we measured plasma F1+2 in uremic patients after intravenous administration of a low molecular weight heparin, which is capable of inhibiting factor Xa, thereby preventing prothrombin conversion into thrombin. The finding that calcium nadroparin caused a marked decrease in the baseline plasma level of F1+2 supports the view that the elevation in F1+2 actually reflects increased *in vivo* conversion of prothrombin into thrombin rather than impaired renal catabolism of this polypeptide.

Weinstein et al. [21] observed high plasma levels of F1+2 in uremic patients on MHD, which were correlated with increased concentrations of fragments of factor VIII coagulation antigen, which may be the result of factor VIII proteolysis by thrombin and/or diminished clearance of proteolyzed factor VIII fragments. Kario et al. [20] have shown that high plasma F1+2 levels are accompanied by factor VII hyperactivity in a series of uremic patients on MHD, suggesting that the increase in F1+2 concentration actually reflects hypercoagulation.

Interestingly, activated factor VII and tissue factor are higher in the plasma of uremic patients on conservative treatment than in healthy controls; both activated factor VII and tissue factor were further increased in uremic patients on MHD [24]. As the interaction between tissue factor and factor VII can trigger the coagulation cascade, it is conceivable that the eventual result is increased thrombin generation.

The significantly higher F1+2 level we have observed in uremic patients on MHD, compared with DT patients, is likely to reflect a more marked activation of the coagulation cascade. Accordingly, in a previous study we found that other molecular markers of hypercoagulation, namely TAT and D-timer, are more elevated in MHD patients than in DT patients [11]. It must be stressed that in our study patients on OHD had significantly lower F1+2 levels than MHD patients, but significantly higher levels than DT patients. Thus long-term hemodialytic treatment may enhance blood coagulation activation.

In addition, within the DT patient group F1+2 correlated positively with creatinine, suggesting a relationship

between the degree of blood coagulation activation and the severity of renal failure. We found no change in plasma F1 + 2 during the course of a single hemodialysis session. Moreover, F1 + 2 levels measured both at the start and at the end of the hemodialysis session from the outlet line were not higher than those measured from the inlet line, providing evidence that extracorporeal circulation per se is not responsible for the F1 + 2 increase. Long-term regular hemodialysis treatment could result in vascular damage, triggering the coagulation cascade, with a consequent plasma F1 + 2 increase.

To conclude, the present study indicates that an accelerated conversion of prothrombin into thrombin by factor Xa, as assessed by the plasma level of F1 + 2, takes place in patients with chronic uremia, to a greater extent in MHD patients than OHD patients and DT patients. The enhanced activation of blood coagulation appears to be related to the severity of renal failure.

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