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Intraperitoneal hypercoagulation and hypofibrinolysis is present in childhood peritonitis

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Abstract An increased rate of obstruction of peritoneal dialysis catheters is observed during peritonitis. Hypercoagulation and hypofibrinolysis may explain this increased occurrence. We studied plasminogen activator inhibitor type 1 antigen (PAI-1), tissue-type plasminogen activator antigen (t-PA), D-dimer (DD), plasmin-α_2-antiplasmin complexes (PAP), and thrombin-antithrombin III complexes (TAT) in 7 children with peritonitis (group A) and 12 children during stable peritoneal dialysis (group B). Albumin, β_2-microglobulin, IgG, and α_2-macroglobulin were measured for baseline transperitoneal protein transport. After a dwell of 6 h with 1.36% Dianeal, dialysate and serum samples were collected. Dialysate to plasma ratios of all proteins were calculated. During peritonitis (group A) TAT was higher: 34.7 versus 22.0 (P=0.01). PAI-1 was increased in group A: 76.5 versus 22.9 (P=0.004). PAP was decreased during peritonitis (group A): 24.9 versus 39.3 (P=0.01). In group A, DD were decreased. 10.8 versus 26.7 (P=0.002). t-PA was similar in both groups (23.7 in group A vs. 27.7 in group B; P=0.26). In both groups TAT, PAI-1, t-PA, PAP, and DD were significantly higher than in baseline transperitoneal transport, suggesting intraperitoneal production. Hypercoagulability and hypofibrinolysis were present during peritonitis compared with the control situation.

Key words Fibrinolysis · Peritonitis · Peritoneal dialysis

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Introduction

In both children and adults peritoneal dialysis is an established therapy for end-stage renal failure. Obligatory for this kind of treatment is a well-functioning peritoneal catheter. Unfortunately one of the major clinical problems is still obstruction of the peritoneal catheter, which is more frequently observed during peritonitis. Hypercoagulability and a relative hypofibrinolysis may explain this increased occurrence. In the literature there is evidence for an imbalance between intraperitoneal coagulation and fibrinolysis during peritonitis in patients on continuous ambulatory peritoneal dialysis (CAPD). Both clinical and in vitro studies in adult peritoneal dialysis patients show evidence of a higher concentration of complexes between thrombin and its inhibitor antithrombin III (TAT complexes), as a marker for hypercoagulation [1–3]. During peritonitis decreased synthesis of tissue-type plasminogen activator antigen (t-PA), the extrinsic activator of plasminogen, and increased synthesis of plasminogen activator inhibitor type I antigen (PAI-1), the main inhibitor of t-PA occur, suggesting impaired fibrinolysis [2].

To investigate the role of the fibrinolytic system we studied PAI-1, t-PA, D-dimer (DD) (the degradation products of fibrin), and plasmin-α_2-antiplasmin complexes (PAP) in both plasma and dialysate in children with peritonitis (group A) and during stable peritoneal dialysis (group B). TAT complexes were measured as markers for ongoing coagulation. The transperitoneal clearances for albumin, β_2-microglobulin (β_2m), IgG, and α_2-macroglobulin (α_2m) were measured as markers for baseline transperitoneal protein transport. In this way

we could control if the measured procoagulant and fibrinolytic factors in the dialysate effluent could be attributed to losses from the intravascular system into the peritoneal cavity.

Patients and methods

Patients

The study group (group A) consisted of 7 children (3 girls, 4 boys) with a median age of 8.2 years (range 2.1–17.2 years). The mean duration of CAPD was 11.1 months (range 2.7–44.0 months). Underlying renal diseases were hemolytic uremic syndrome, renal hypoplasia/dysplasia, cystinosis, posterior urethral valves, congenital nephrotic syndrome, focal glomerulosclerosis, and tubulointerstitial nephritis. Histopathology of the patient diagnosed as congenital nephrotic syndrome revealed normal-appearing glomeruli and slight tubular dilatation. Peritonitis was clinically diagnosed as cloudy dialysate. The causal organisms isolated were: *Staphylococcus aureus* (2), *Streptococcus*, *Staphylococcus epidermidis*, *Acinetobacter*, and unknown. The control group (group B) consisted of 12 stable children (3 girls, 9 boys) with a median age of 10.8 years (range 2.4–15.9 years). They had been treated by peritoneal dialysis for a mean period of 15.8 months (range 3.8–36.3 months). Causes of end-stage renal disease were posterior urethral valves (4), focal glomerulosclerosis (3), renal hypoplasia/dysplasia (2), hemolytic uremic syndrome, Henoch-Schönlein nephritis, and interstitial nephritis.

Methods

After a dwell of 6 h with dialysis fluid containing 1.36% glucose (Dianeal 1.36%, Baxter, Deerfield, Ill., USA), dialysate was collected and blood samples were taken from all patients. The samples were added to citrate theophylline adenosin dextrose (CTAD) or citrate media for measurement of PAI-1, t-PA (CTAD) and DD, PAP, TAT (citrate). In the study group, during peritonitis, samples were taken between 24 and 48 h after the start of treatment of peritonitis. Dialysate and blood samples were centrifuged to remove cells and debris, and stored at –70° C until analysis. Albumin, IgG, and α_2m were quantified by nephelometry. PAI-1, tPA, DD, PAP, TAT, and β_2m were measured by enzyme-linked immunosorbent assay. For each individual patient the dialysate to plasma (D/P) ratio of all measured parameters was calculated. The protocol was approved by the medical ethics committee of the University Hospital Nijmegen. Informed consent was obtained from the parents and older children.

Statistical analysis

All data are expressed as median and reference intervals (5th and 95th percentiles) are given. Statistical comparisons between the two groups were performed using the Mann-Whitney U-test, and the 95% confidence interval for the difference in medians was calculated [4]. For each individual patient, least squares regression was performed using the results of the four non-procoagulant or fibrinolytic proteins. The log base 10 of the dialysate/serum ratio ($\log_{10}$ [R]) was used as the dependent variable and the log base 10 of the molecular weight ($\log_{10}$ [W]) as the independent variable. The assumption of a linear relationship between $\log_{10}$ [R] and $\log_{10}$ [W] was checked with the Pearson correlation coefficient. With these regression lines a predicted D/P ratio was determined for every procoagulant and fibrinolytic factor in each patient. Predicted and measured D/P ratios were subtracted. The differences were tested for significance using a modified *t*-test, taking into account the inaccuracy of the prediction [5]. A full description of this modification is given in the Appendix. Any *P* values less than 0.05 were considered significant. All calculations were done on a logarithmic scale and then transformed.

Results

The D/P ratios of TAT, PAI-1, t-PA, PAP, and DD of both groups are given in Table 1. The D/P ratio of TAT is significantly higher in group A, indicating a higher rate of intraperitoneal coagulation during peritonitis than stable peritoneal dialysis. The D/P ratio of PAP and DD is significantly lower in group A, indicating impaired fibrinolysis during peritonitis. The D/P ratio of PAI-1, an inhibitor of fibrinolysis, is significantly higher in the peritonitis group, reflecting impaired fibrinolysis. The D/P ratio of t-PA is lower during peritonitis, but this result is not significant.

In plasma the concentrations of TAT, PAI-1, and DD do not differ in stable peritoneal dialysis and during peritonitis. In both groups they are within the normal range of our reference values (Table 2), except for the concentration of PAI-1 during peritonitis. In the peritonitis group, plasma t-PA is significantly increased (P=0.03) and PAP complexes are significantly decreased (P=0.01) compared with the control situation, but in both groups they are in the normal range. The D/P ratios of TAT [kilodaltons 130.5 (kDa)], PAI-1 (52 kDa), t-PA (68 kDa), PAP (153 kDa), and DD (180 kDa) of 1 patient are plotted against molecular weight on a double-logarithmic scale in Fig. 1. The expected relationship between molecular weight and D/P ratio, based on measurements of β_2m (11.8 kDa), albumin (69 kDa), IgG (160 kDa), and α_2 m (820 kDa), is indicated as a reference line. The correlation coefficients for the relationship between $\log_{10}$ [R] and $\log_{10}$ [W] were smaller than

Table 1 Dialysate to plasma ratios (D/P) (percentage) of the measured procoagulant and fibrinolytic factors in 7 patients with peritonitis (group A) and 12 patients during stable perotineal dialysis (group B) [a]

	Group A	Group B	95% CI for the difference B–A	*P* *
TAT	38.1 (22.1, 84.3)	22.0 (16.7, 32.7)	–28.7, –2.3	0.01
PAI-1	77.3 (23.1, 119.2)	22.9 (14.1, 42.3)	–66.8, –27.9	0.004
t-PA	23.8 (19,2, 40.7)	27.7 (22.9, 60.7)	–2.6, 12.3	0.26
PAP	25.6 (18.5, 60.9)	39.3 (29.0, 66.0)	3.8, 32.4	0.01
DD	9.5 (6.0, 23.2)	26.7 (18.9, 40.8)	9.4, 20.5	0.002

CI, Confidence interval; TAT, thrombin-antithrombin III complexes; PAI-1, plasminogen activator inhibitor type I antigen; +-PA, tissue-type plasminogen activator antigen; PAP, plasmin-α_2-antiplasmin complexes; DD, D-dimer

* Statistical comparisons are performed using the Mann-Whitney U test and the 95% CI for the difference in medians is calculated

[a] Values are expressed as median and reference intervals (5th and 95th percentiles) are shown

Table 2 Coagulation- and fibrinolysis-related factors in plasma of 7 patients with peritonitis (group A) and 12 patients during stable peritoneal dialysis (group B) compared with the reference values [a]

	Group A	Group B	95% CI for the difference B-A	Reference values
TAT (ng/ml)	8.7 (6.8, 12.1)	7.0 (4.1, 10.7)	–4.3, 0.3	2–10
PAI-1 (ng/ml)	12.8 (3.9, 21.3)	6.0 (3.8, 11.2)	–9.9, 0.8	2–10
t-PA (ng/ml)	18.5 (14.5, 21.8)	13.7 * (7.1, 18.7)	–8.3, –0.6	10–20
PAP (nmol/l)	3.1 (2.7, 4.6)	5.2 * (3.0, 8.0)	0.4, 3.1	3–8
DD (µg/ml)	48.0 (34.2, 61.3)	52.4 (30.6, 68.2)	–9.2, 15.2	30–75

* Significantly different

[a] Values are expressed as median and reference intervals (5th and 95th percentiles) are shown. Statistical comparisons are performed using the Mann-Whitney U test and the 95% CI for the difference in medians is calculated. The reference values are expressed as range

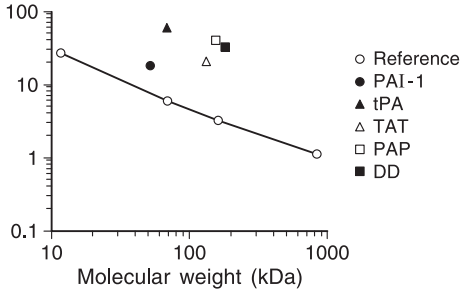

Fig. 1 The dialysate/plasma (*D*/*P*) ratios of thrombin-antithrombin III complexes (*TAT*), plasminogen activator inhibitor type I antigen (*PAI-1*), tissue-type plasminogen activator antigen (*t-PA*), plasmin-α_2-antiplasmin complexes (*PAP*), and D-dimer (*DD*) (in percentage) versus molecular weight [kilodaltons (kDa)] on a double-logarithmic scale of 1 patient during stable peritoneal dialysis. The expected relationship between molecular weight and ratio, based on measurements of β_2-microglobulin, albumin, IgG, and α_2-macroglobulin, is indicated as a reference line

–0.96 in all patients. For 2 patients the *P* values were above 0.05 (0.057 and 0.16), but the regression lines for these patients were each based on only three points. The remaining patients all had *P* values <0.05. The D/P ratios of all measured procoagulant and fibrinolytic antigens are higher than the reference line. These findings indicate local intraperitoneal production of TAT, PAI-1, t-PA, PAP, and DD. The 1 patient shown is representative for all patients.

Table 3 shows the predicted ratios of the procoagulant and fibrinolytic factors based on their molecular weight in both groups. The quotient of the measured and predicted ratios show that for every procoagulant and fibrinolytic antigen the measured D/P ratio was significantly higher than predicted based on molecular weight (*P*<0.001).

Table 3 The predicted D/P ratios of TAT, PAI-1, t-PA, PAP, and DD (percentage) based on molecular weight, during peritonitis (group A) and during stable peritoneal dialysis (group B). The quotients of the measured and predicted ratios are tested using a modified *t*-test [5] [a, *]

	Predicted ratio group A	Quotient measured/ predicted D/P ratio	Predicted ratio group B	Quotient measured/ predicted D/P ratio
TAT	3.2 (1.1, 14.6)	8.1 (5.8, 46.7)	1.9 (1.2, 5.2)	13.1 (3.2, 19.0)
PAI-1	7.3 (3.7, 23.4)	9.3 (3.1, 30.3)	4.4 (2.8, 11.1)	5.4 (1.9, 11.6)
t-PA	5.7 (2.6, 20.4)	5.8 (1.1, 9.3)	3.5 (2.2, 8.9)	8.1 (2.7, 17.5)
PAP	2.8 (0.9, 13.4)	12.6 (1.9, 31.6)	1.6 (1.0, 4.6)	23.4 (8.4, 52.1)
DD	2.4 (0.7, 12.3)	5.9 (0.8, 13.2)	1.4 (0.8, 4.0)	21.6 (5.6, 35.8)

* For every procoagulant and fibrinolytic factor the measured D/P ratio was significantly higher than the predicted D/P ratio (*P*<0.001)

[a] Values are expressed as median and reference intervals (5th and 95th percentiles) are shown

Discussion

Intraperitoneal hypercoagulation and fibrinolysis took place in our patients, as shown by the significantly higher than expected D/P ratios of TAT, PAI-1, t-PA, PAP, and DD on the basis of their molecular weights. These results exclude the possibility that the high concentrations of coagulation- and fibrinolysis-related factors in the dialysate are mainly the result of transport from plasma into the perioneal cavity, as shown for other factors [1, 5].

During peritonitis hypercoagulation appears even more marked, as illustrated by a significantly higher D/P ratio of TAT in our study group compared with the control group. Simultaneously, fibrinolysis is considerably impaired in the peritonitis group compared with the stable peritoneal dialysis group, as shown by a significantly decreased D/P ratio of PAP, leading to a significantly decreased D/P ratio of DD. This hypofibrinolysis is caused by a higher intraperitoneal production of PAI-1 during peritonitis, as shown by an increased D/P ratio of PAI-1. The D/P ratio of t-PA is also decreased during peritonitis, but this result is not significant. These results are in agreement with the reported imbalance between intraperitoneal coagulation and fibrinolysis during peritonitis in the literature [1, 2].

In our study the plasma concentrations of TAT, PAI-1, and DD do not differ between stable peritoneal dialysis and peritonitis. In both groups they are in the normal range for the reference values, except for a slightly higher PAI-1 plasma concentration in group A. We could not confirm the increased TAT and DD serum levels that

both Goedde et al. [1] and Gries et al. [37] found in their patients on stable peritoneal dialysis.

The imbalance between intraperitoneal coagulation and fibrinolysis can explain the visible fibrin clots that appear more marked during peritonitis and may obstruct the peritoneal catheter. The mechanism of impaired fibrinolysis during peritonitis may be explained by the elegant studies of Holmdahl et al. [6] who showed that under normal conditions t-PA is present in the mesothelium and substantially reduced in inflammation. PAI-1 is present in both mesothelium and submesothelial capillary vascular walls, and its expression is intensified and widely distributed in the submesothelial tissue during peritonitis.

Heparin, by increasing the conjugation rate of antithrombin III with thrombin, and thereby inactivating the effect of thrombin, might prevent fibrin formation [7, 8]. Addition of 500 U heparin/l of dialysate is shown to be sufficient to inhibit intraperitoneal fibrin formation without a systemic anticoagulant effect [9, 10]. Recently Nadig et al. [8] concluded from their studies that heparinization is effective in patients with low DD levels in the dialysate at the time of peritonitis.

Fibrinolytic therapy with urokinase can be applied when the fibrin deposits have already caused catheter obstruction [11]. In 6 of 8 patients in whom we measured local production of DD in the peritoneal catheter after urokinase therapy, we found a rise of DD, suggesting enhanced fibrin degradation (unpublished data).

In summary, our results show that in children, both on stable peritoneal dialysis and during peritonitis, TAT, PAI-1, tPA, PAP, and DD are produced intraperitoneally. During peritonitis in children, hypercoagulation and hypofibrinolysis occur compared with stable peritoneal dialysis, which may contribute to fibrin deposits and subsequent obstruction of the peritoneal catheter.

Appendix

For each individual k, let:
W_i be the molecular weight of non-procoagulant and fibrinolytic factor i; W_f be the molecular weight of procoagulant and fibrinolytic factor f; R_i be the measured D/P ratio of non-procoagulant and fibrinolytic factor i; R_f be the measured D/P ratio of procoagulant and fibrinolytic factor f. Of N individuals, for each individual k, the regression line of $\log_{10}$ [R] and $\log_{10}$ [W] was fitted: Y=a+bX, where Y=$\log_{10}$ [R] and X=$\log_{10}$ [W]. The regression line was based on $n_k$complete pairs of measurements ($\log_{10}$ [W_i], $\log_{10}$ [R_i]). Let X_f be $\log_{10}$ [W_f], X_i be $\log_{10}$ [W_i], Y_f^m be the measured $\log_{10}$ [R_f], Y_f^p be the predicted $\log_{10}$ [R_f], and $d_f = Y_f^m \cdot Y_f^p$.

The variance of Y_f^m is assumed to be the same for all procoagulant and fibrinolytic factors and equal to σ^2, the variance in the determination of the non-procoagulant and fibrinolytic factors. The variance of Y_f^p can be calculated with the following formula [12]:

$$\sigma^2\left[\frac{1}{n_k} + \left(X_f - \overline{X}_k\right) \Big/ \sum_i \left(X_i - \overline{X}_k\right)^2\right]$$

Let $\overline{X}_k$ be the mean $\log_{10}$ [W] of the non-procoagulant and fibrinolytic factors of individual k, then the variance of d_f is the sum of the variances of Y_f^m and Y_f^p:

$$\sigma^2\left[1 + \frac{1}{n_k} + \left(X_f - \overline{X}_k\right) \Big/ \sum_i \left(X_i - \overline{X}_k\right)^2\right]$$

Let $\overline{d}_f = \frac{1}{N}\sum_{k=1}^{N} d_{kf}$, the mean of all individual d_f, then $\left(\overline{d}_f\right) = \frac{1}{N_2}\sum_{k=1}^{N} \mathrm{var}(d_{kf})$. Then $\frac{\overline{d}_f}{\mathrm{SE}\left(\overline{d}_f\right)}$ has a t distribution with $\sum_{k=1}^{N} df_k$ degrees of freedom where $\mathrm{SE}(\overline{d}_f)$ is an estimation for $\sqrt{\mathrm{var}\left(\overline{d}_f\right)}$ obtained by estimating σ^2 with the pooled mean squared error and where $df_k = n_{k-2}$.

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