Intraperitoneal heparin reduces peritoneal permeability and increases ultrafiltration in peritoneal dialysis patients

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Abstract

Background. Patients on long-term treatment with peritoneal dialysis (PD) suffer from increasing peritoneal permeability and loss of ultrafiltration as a result of persistent inflammation, which may be triggered by bioincompatible dialysis fluids. Heparins have anti-inflammatory and anticoagulant properties. We have examined the effect of intraperitoneal (IP) low-molecular weight heparin (tinzaparin) on peritoneal permeability and ultrafiltration in PD patients.

Methods. By means of a double-blinded cross-over design, 21 PD patients were randomized to receive either placebo or tinzaparin intraperitoneally once a day for two treatment periods of 3 months, separated by a wash-out period. The effect of heparin on peritoneal permeability and ultrafiltration was assessed using the 4 h standard peritoneal equilibration test.

Results. IP tinzaparin reduced significantly the dialysate-to-plasma ratios (D/P) of creatinine \((P < 0.01)\), urea \((P < 0.01)\) and albumin \((P < 0.05)\). In addition, the ratio of glucose concentration in dialysate at 4 h dwell to that of 0 h dwell \((D_4/D_0)\) was increased \((P < 0.05)\) along with an increase in ultrafiltration volume \((P < 0.05)\).

Conclusions. IP tinzaparin reduces peritoneal permeability to small solutes and increases ultrafiltration in PD patients.

Keywords: heparin; intraperitoneal administration; peritoneal dialysis; peritoneal transport; ultrafiltration

Introduction

In peritoneal dialysis (PD) the mesothelium is exposed to bioincompatible solutes imposing a state of chronic low-grade inflammation. Such a low-grade inflammatory state may in long-term PD cause functional deterioration of the peritoneal membrane with increasing peritoneal permeability for small solutes and reduced ultrafiltration [1,2]. This may be clinically important as patients with high/high average (H/HA) transport characteristics measured by peritoneal equilibration tests (PETs) [3] have poor prognosis regarding technical survival as well as patient mortality compared with low/low average (L/LA) transporters [4–6].

Several attempts to modify outcome of dialysis with intraperitoneal (IP) administration of glycosaminoglycans have been reported [7–10]. However, the results have been contradictory, and no placebo-controlled studies have been reported regarding the possible effect of IP administration of glycosaminoglycans in humans. In the present randomized, double-blinded, placebo-controlled cross-over study we administered IP glycosaminoglycans in the form of a low-molecular weight heparin (i.e. tinzaparin) to patients on PD. Our results provide evidence that IP administration of 4500 Anti-Xa IU tinzaparin in morning dialysis bags reduces peritoneal permeability, and increases ultrafiltration, without adding to bleeding risk.

Subjects and methods

Patients and study design

Patients were recruited from November 2001 to April 2002 at the Department of Nephrology, Ribe County Hospital in Esbjerg/Varde, Denmark. Inclusion criteria were uncomplicated PD for at least 3 months, and continuous ambulatory peritoneal dialysis (CAPD) or automated peritoneal dialysis (APD) with at least one daily daytime dialysis bag. Exclusion criteria were age below 18 years, infectious peritonitis within the last 2 months, known thrombophilia, treatment with oral anticoagulants, systemic infection and pregnancy. All fertile women were urged to use contraceptives. Twenty-one patients entered the study. All patients gave written informed consent. Patients developing peritonitis, systemic infections or those transferred to other dialysis modalities during the...
trial were censored (drop-outs). The study protocol was approved by the local scientific ethics committee (approval number 2310-01) and the Danish National Board of Medicine (approval number 2612-1798). The study was conducted as a double-blinded randomized cross-over trial with two treatment periods of 3 months, separated by a 1 month wash-out period. Patients injected 4500 Anti-Xa IU tinzaparin (LEO Pharmaceutical Products, Ballerup, Denmark) or placebo (isotonic saline) into their morning dialysis bag prior to dialysis every day during the treatment periods, whereas no trial medication was used during the wash-out period. The study lasted 7 months in total for each patient completing the trial. Before commencing the trial, the patients had been through a thorough training programme to inject the trial medication into their morning bags under sterile conditions.

A total of four standard PETs (according to Twardowski et al. [3]) were performed on each patient (one test before and one test after each treatment period). This test evaluates the peritoneal permeability to small solutes. The dialysate used in every PET was 2.27% (w/v) glucose (Dianeal PD4; Baxter A/S, Allerod, Denmark). Dialysate-to-plasma ratios (D/P) of creatinine, urea and albumin were calculated from the 4 h dialysate values and 2 h plasma values. The permeability for glucose was estimated as the ratio between the dialysate glucose concentration at 4 h to that of 0 h dwell (D4/D0). The ultrafiltration volume was measured as the total drainage volume after 4 h dwell minus 2000 ml (input volume). All samples (plasma and dialysate) were stored in aliquots of 1.2 ml at minus 65°C until analysis.

**Variables**

The Cobas Integra 800 (Roche Diagnostics GmbH, Mannheim, Germany) was used to analyse the concentrations of the following compounds in plasma and dialysate: creatinine (enzymatic method, which is not affected by glucose in the dialysate [11]); urea (enzymatic method); plasma albumin (modified bromcresol green method); and glucose (enzymatic method). All samples (plasma and dialysate) were measured using assays intended for plasma, except for dialysate albumin, which was measured using the immunoturbidimetric assay (340 nm) for urine albumin (with anti-albumin T antiserum (rabbit) specific for human albumin).

Platelet concentrations and erythrocyte volume fractions were analysed on an Advia 120 (Bayer Diagnostics Norden A/S, Kongens Lyngby, Denmark) haematology analyser. The activated partial thromboplastin time (APTT) in plasma was analysed on a Behring Coagulation System (Dade Behring Marburg GmbH, Marburg, Germany) using the PTT automated assay (Diagnostica Stago S.A., Asnières, France). The concentrations of low-molecular weight heparin (Anti-Xa) in plasma and dialysate were measured on an ACL 7000 (Instrumentation Laboratories SpA, Milano, Italy) using a chromogenic assay (Stachrom Heparin from Diagnostica Stago S.A.), with a sensitivity of 0.1 Anti-Xa U/ml.

**Statistical analysis**

The statistical software used was SigmaStat for Windows (version 2.03, SPSS Inc.). Per protocol statistics were used unless otherwise stated. Non-parametric statistics were used for all variables, and the Wilcoxon signed-ranks test was used to compare variables between treatment periods. When comparing groups of patients, the Fisher exact test and the Mann–Whitney U-test were used for categorical and numerical data, respectively. The Spearman rank order correlation was used to test associations between variables. The level of significance was set to $P < 0.05$. All data are presented as median (25th and 75th percentiles in brackets), unless otherwise stated.

**Results**

Table 1 shows the baseline characteristics of the patients. Ten patients did not complete the trial. One patient died from severe ischaemic heart disease, one patient suffered from sepsis (without coexistent peritonitis), one patient was transferred to another dialysis modality, and seven patients suffered from peritonitis (three patients while using placebo, three patients while using tinzaparin, and one patient during the wash-out period). The 11 patients thus completing the trial constitute the basis for the per protocol analysis. There were no significant differences between the baseline characteristics of the patients dropping out and the patients completing the study ($P > 0.1$ for all parameters and variables). The mean D/P creatinine in the 11 patients completing the trial was 0.76±0.13 (SD), thus 55% were H/HA transporters [3]. The total duration of the trial lasted 102 patient-months (drop-outs included).

There were no bleeding complications, and the platelet count and erythrocyte volume fraction did not change within the treatment periods (Table 2). A period effect (i.e. that the difference between tinzaparin and placebo was greater in patients commencing the trial with tinzaparin than in the patients commencing the trial with placebo) was only observed in plasma

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics of the PD patients</th>
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<tbody>
<tr>
<td>Included (n=21)</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Males (%)</td>
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<tr>
<td>Renal disease</td>
</tr>
<tr>
<td>Diabetic nephropathia (%)</td>
</tr>
<tr>
<td>Chronic glomerulonephritis (%)</td>
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<tr>
<td>Hypertensive nephropathia (%)</td>
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<tr>
<td>Poly cystic renal disease (%)</td>
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<tr>
<td>Other renal diagnosis (%)</td>
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<tr>
<td>Arterial thromboembolic disease (%)</td>
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<tr>
<td>Time on PD (months)</td>
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<tr>
<td>Treatment with</td>
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<tr>
<td>Erythropoietin (%)</td>
</tr>
<tr>
<td>Beta-blockers (%)</td>
</tr>
<tr>
<td>Statins (%)</td>
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<tr>
<td>ARB (%)</td>
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<tr>
<td>CAPD (i.e. not APD) (%)</td>
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</tbody>
</table>

*The 11 patients completing the trial form the basis for the per protocol statistical analysis in the text.

AR, angiotensin receptor blockers.
albumin. There were, however, no carry-over effects in any of the parameters and variables investigated in this study, and all parameters and variables before tinzaparin treatment were not significantly different from the parameters and variables before placebo treatment.

IP tinzaparin induced a significant reduction in D/P creatinine \( (P < 0.01) \) and D/P urea \( (P < 0.01) \) compared with placebo (Figure 1). The reduction in the median value of D/P creatinine was 16%. While the D/P albumin was significantly reduced after tinzaparin treatment compared with placebo \( (P < 0.05) \), the plasma albumin did not change during the trial (data not shown).

D\(_4\)/D\(_0\) glucose and ultrafiltration both increased on tinzaparin treatment compared with placebo but did not reach statistical significance. However, when comparing the values after tinzaparin treatment with the values before the treatment, significant increases in

### Table 2. Safety markers in blood during the trial \((n = 11)\)

<table>
<thead>
<tr>
<th></th>
<th>Before placebo</th>
<th>After placebo</th>
<th>Before tinzaparin</th>
<th>After tinzaparin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count ((\times 10^9/\text{l}))</td>
<td>273 (232; 297)</td>
<td>276 (256; 325)</td>
<td>254 (245; 323)</td>
<td>257 (242; 268)</td>
</tr>
<tr>
<td>EVF (l)</td>
<td>0.37 (0.36; 0.39)</td>
<td>0.39 (0.35; 0.41)</td>
<td>0.40 (0.37; 0.41)</td>
<td>0.36 (0.35; 0.40)</td>
</tr>
<tr>
<td>APTT (relative time)</td>
<td>0.99 (0.93; 1.09)</td>
<td>0.99 (0.91; 1.14)</td>
<td>0.98 (0.91; 1.13)</td>
<td>0.97 (0.93; 1.05)</td>
</tr>
<tr>
<td>Anti-Xa (IU/ml)</td>
<td>0.1 (0.1; 0.1)</td>
<td>0.1 (0.1; 0.1)</td>
<td>0.1 (0.1; 0.1)</td>
<td>0.1 (0.1; 0.1)</td>
</tr>
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</table>

EVF, erythrocyte volume fraction. Values are median values with the 25th and 75th percentiles in brackets.

Fig. 1. The evolution of D/P creatinine (A), D/P urea (B), D/P albumin (C) and D\(_4\)/D\(_0\) glucose (D) during the trial \((n = 11)\). Boxes represent median values, and error bars represent the 75th (top) and 25th (bottom) percentiles. NS, not significant.
D₄/D₀ glucose and ultrafiltration were observed (P < 0.05 and P < 0.05, respectively), whereas placebo had no such effect (Figures 1 and 2).

When stratifying the patients into transporter subgroups (i.e. H/HA and L/LA) according to the initial PET, similar effects of IP tinzaparin on D/P creatinine, D/P urea, D/P albumin and ultrafiltration were observed in both subgroups. The increase in D₄/D₀ glucose on IP tinzaparin treatment was, however, higher in the H/HA transporters compared with the L/LA transporters.

As shown in Table 2, the APTT and Anti-Xa in plasma were unaffected by tinzaparin treatment. In overnight dialysate, Anti-Xa was only detectable in three patients after tinzaparin treatment (values were 0.47, 0.46 and 0.33 IU/ml, respectively), and the overall median value was not significantly different from the values after placebo treatment (Anti-Xa was undetectable).

Discussion

In this study we have demonstrated that IP tinzaparin causes a significant reduction in D/P creatinine, D/P urea and D/P albumin associated with a significant increase in D₄/D₀ glucose and ultrafiltration in patients on PD. This effect is not dependent on initial stratification according to transporter subgroup status.

The effects of IP heparin-like substances in PD have previously been reported with inconsistent results. Thus, it has been reported that IP glycosaminoglycans may enhance D/P creatinine [7,8], decrease peritoneal protein loss [8], increase ultrafiltration [10] or that it may have no effect on D/P creatinine, protein-loss or ultrafiltration [9]. This inconsistency can be explained by inadequate study designs such as short intervention periods and lack of randomized placebo groups. In addition, we have in the present study used a more accurate method for the determination of creatinine in plasma and dialysate [11].

The mechanism by which continuous exposure of the peritoneum to bioincompatible solutions causes inflammation and impaired peritoneal membrane function is poorly understood. However, during tissue injury mesothelial and endothelial cells produce cytokines (e.g. tumour necrosis factor alpha and interleukins), which cause a release of tissue factor from monocytes and macrophages. Tissue factor increases local thrombin generation, which activates platelets and endothelium to produce growth factors thus enhancing neoangiogenesis and local fibrosis [12–14]. Neoangiogenesis causes an overall increase in peritoneal vascular surface area, which is closely related to increased peritoneal permeability [15]. Besides reducing thrombin generation, heparins can induce an increase in tissue factor pathway inhibitor [16], which inhibits tissue factor activity. Hence, the anti-inflammatory properties of heparins may take place through a reduction of thrombin generation, and through inhibition of the tissue factor pathway of coagulation. These effects would reduce the local release of growth factors and cytokines, thereby diminishing local inflammation. As such, heparins may act on the peritoneal membrane, thereby preventing further damage from bioincompatible solutions. Our data indicate that heparins have an active modulating effect on the peritoneal membrane, as the D/P creatinine, D/P urea and D/P albumin were reduced after IP tinzaparin treatment compared with the values obtained before and after placebo treatment.

The bioincompatibility of peritoneal solutes can be understood as the ability of the peritoneal solutes to elicit unwanted pathophysiological responses in target organs (e.g. increased peritoneal permeability, reduction of peritoneal ultrafiltration and others). In the present study it was demonstrated that IP tinzaparin decreases the peritoneal permeability to small solutes and increases ultrafiltration, thus reducing the level of bioincompatibility of the peritoneal solutes used in the trial. In addition, biocompatibility markers such as von Willebrand factor and soluble intracellular adhesion molecule I were undetectable in overnight dialysates of our patients (data not shown). This does not indicate that the dialysates used by our patients were biocompatible, but rather that we failed to demonstrate the level of bioincompatibility of the dialysates.

Obviously the results presented in this paper should be interpreted with caution as the present study is based on a relatively small sample size. Owing to the crossover design, however, the present study is the largest clinical study performed to date. Another drawback of this study is the high incidence of peritonitis. Seven cases of peritonitis during the 102 patient-months were encountered. Treatment with IP tinzaparin did not cause a higher incidence of peritonitis than treatment with IP placebo. Thus, the most likely explanation for the peritonitis rate was the injection procedure of the trial medication. The syringes used had short needles
(1.2 cm), which could have caused difficulties in maintaining sterile conditions, despite careful training in aseptic injection techniques.

The modifying effects of heparin on peritoneal permeability and ultrafiltration observed in the present study should be put into perspective with observations suggesting that mortality is higher in H/HA transporters compared with L/LA transporters [4–6]. The inadequacy to mount sufficient ultrafiltration in H/HA transporters leads to fluid overload, hypertension, congestive heart failure and death. Treatment with IP heparins might enhance fluid elimination not only in H/HA transporters but also in L/LA transporters by increasing ultrafiltration and decreasing peritoneal permeability, thus preventing the aforementioned complications. Increased ultrafiltration leads to reduced hypertension, a known risk factor for cardiovascular disease and fluid retention. Consequently, the need for hypertonic dialysate solutions diminishes, thereby decreasing the IP inflammation. In addition, decreased glucose absorption could reduce the formation of advanced glycation end-products in plasma, as demonstrated by Mizuiri et al. [9]. Advanced glycation end-products are claimed to increase peritoneal permeability and to be involved in vascular sclerosis and neoangiogenesis [13,17,18].

In conclusion, we have demonstrated that IP tinzaparin decreases D/P creatinine, D/P urea and D/P albumin in patients on PD. In addition, IP tinzaparin also leads to decreased glucose absorption with a subsequent increase in ultrafiltration without adding to bleeding risk.

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Conflict of interest statement. None declared.

References


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