Effects of intraperitoneal heparin on peritoneal transport in a chronic animal model of peritoneal dialysis

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Abstract

Background. Heparin has anti-inflammatory effects and is often added to the peritoneal dialysis fluid to prevent fibrin formation. Conjugation of heparin to the surface of biomaterials has been shown to improve its biocompatibility. In this study, we describe for the first time an experimental chronic peritoneal dialysis model with repeated dwell studies in non-uraemic rats and evaluate the effect of addition of heparin to glucose-based peritoneal dialysis fluid on peritoneal fluid and solute transport.

Methods. Wistar male rats, weighing 340 ± 15 g, with implanted peritoneal catheters were infused during 1 month, twice per day with 20 ml of Dianeeal 1.36% + antibiotics (AB; n = 10) or Dianeeal 1.36% + antibiotics + heparin 2500 U/l (HAB; n = 9). After 10 (DS 1) and 30 days (DS 2), a dwell study was performed in rats with free access to drinking water, by infusing 30 ml of Dianeeal 3.86%. Dialysate samples were obtained at 0, 2, 30, 60, 120 and 240 min. Blood samples were drawn before and at the end of the dwell. Radiolabelled serum albumin was used as macromolecular volume marker.

Results. Peritoneal volumes during DS 1 were significantly greater for the HAB group as compared with the AB group. No differences in ultrafiltration were found during DS 2 for HAB vs AB. However, peritoneal volumes were significantly higher for DS 2 compared with DS 1 in the AB group. The amount of glucose absorbed over time did not differ between the solutions, while fluid absorption tended to be lower in the HAB group.

Conclusions. Heparin may improve peritoneal fluid transport possibly due to better healing and reduced peritoneal inflammation as shown in this novel animal model of chronic peritoneal dialysis with repeated dwell studies.

Keywords: animal model; chronic peritoneal dialysis; heparin; peritoneal transport; repeated dwell studies; ultrafiltration

Introduction

Peritoneal permeability is a prognostic factor for the outcome of long-term peritoneal dialysis, and kinetic modelling in peritoneal dialysis can provide a tool for patient management. Animal models of peritoneal dialysis have been used to evaluate the mechanisms that regulate transperitoneal transport [1]. At present, no one ideal experimental in vivo model for studying changes in peritoneal permeability exists. It seems that the optimal animal experiments on chronic peritoneal dialysis are those done on unrestrained, awake animals with free access to water and food [2]. Some of the results found in ‘acute studies’, lasting several hours and performed on anaesthetized animals, may not be the same as those observed in patients maintained on continuous ambulatory peritoneal dialysis (CAPD). Therefore, one should attempt to exclude the potential artefacts induced by anaesthesia [3] and acute exposure to dialysis solutions in vivo studies.

Heparin is a sulfated mucopolysaccharide comprising D-glucuronic acid and D-glucosamine, with a molecular weight between 6000 and 30 000 Da (mean 15 000). Heparin has more sulfate radicals than other glycosaminoglycans and has a number of immunomodulatory and anti-inflammatory activities [4,5]. It is often added to the peritoneal dialysis fluid to prevent fibrin formation [6]. In this study, we established an experimental chronic peritoneal dialysis model for repeated dwell studies in non-uraemic rats and evaluated the effect of heparin used as an additive to Dianeeal 1.36% on peritoneal permeability.

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Materials and methods

Experiments were performed on male Wistar rats weighing 340 ± 15 g (n = 19). On day 1, under anaesthesia (medetomidin and midazolam, i.m.), omentectomy was performed and a peritoneal catheter was implanted into the peritoneal cavity in all animals. The method of catheter implantation and peritoneal dialysis in rats was described previously [7,8]. During the following 9 days, rats were infused once daily with a gradually increasing volume (10–20 ml) of Dianeeal 1.36% (AB; n = 10) or Dianeeal 1.36% + standard heparin (Heparin Leo, Lövens Kemiske Fabrik, Ballerup, Denmark) 2500 U/l (HAB; n = 9). On day 10, and for the following 20 days, test dialysis solutions were instilled (20 ml) twice daily and the infused solution was allowed to be absorbed from the peritoneal cavity. All dialysis solutions were supplemented with antibiotics, netilmicin 5 mg/l and cefuroxim 60 mg/l.

Dwell study

On the 10th (DS 1) and 30th (DS 2) days of the experiment, a 4 h dwell study was performed by infusing 30 ml of Dianeeal 3.86% in awake animals with free access to drinking water. The dialysis solution was prepared with radiolabelled albumin [200 kBq of 125I-labelled human serum albumin (RISA); Isopharma AS, Kjeller, Norway]. A priming dose of 0.2 g/l of human albumin was added in Dianeeal 3.86% to minimize the adherence of tagged albumin to the surface of the plastic material. Dialysate samples (0.7 ml) were obtained at 0, 2, 30, 60, 120 and 240 min. After 240 min, the dialysate was drained by gravity for 5 min. The abdominal cavity was then rinsed for 1 min with 20 ml of fresh Dianeeal 1.36% (without RISA) to provide data for calculation of the residual volume at 240 min. Blood samples were drawn before and at the end of the dwell from the tail artery. The experimental study was approved by the Animal Ethical Committee of the Karolinska Institute at Huddinge Hospital.

The radioactivity of the dialysate (0.1 ml) and blood samples (0.1 ml of plasma) was analysed in a Gamma Counter (Packard Instrument Company, Meriden, CT). Dialysate concentrations of glucose (hexokinase method) were analysed using a Monarch™ 1000 autoanalyser (Instrumentation Laboratory, Lexington, MA). Intraperitoneal dialysate volume was estimated from the dilution of RISA with corrections made for the elimination of RISA from the peritoneal cavity and the sample volumes [9]. In addition, at the end of the dwell, the dialysate volume was measured directly. The peritoneal fluid absorption rate was estimated as the coefficient of RISA elimination from the peritoneal cavity, KE (ml/min), using a formula reported previously [10]. Since only a very small amount of free iodine was found in the RISA solution [11], we did not take the free iodine into account in the KE calculation. The computer program Pertran (Baxter Novum) was used for the evaluation of peritoneal dwell studies, and can be found at http://www.ibb.waw.pl/~peritone.

Statistical analysis

Statistical analysis of the data was performed with the Mann–Whitney rank sum test or with a two-way analysis of variance with repeated measurements (ANOVA) using a post-hoc Scheffe test, as appropriate. Data are presented as mean ± standard deviation and differences were considered statistically significant with P < 0.05.

Results

Peritoneal volumes estimated from the dilution of RISA during DS 1 were significantly greater for the heparin-treated group (HAB) at 30 (P < 0.001), 60 (P < 0.001) and 120 min (P < 0.02) as compared with the AB group (Table 1). The mean volume of drained dialysate at the end of DS 1 was 38.1 ± 3.5 ml for the HAB group vs 37.0 ± 2.1 ml for the AB group (P = 0.133). After 30 days of peritoneal dialysis in rats, no difference in net ultrafiltration was found during DS 2 with HAB vs AB (Table 1).

However, in the AB group, peritoneal volumes were significantly higher at DS 2 than at DS 1, at 30 (P < 0.0001), 60 (P < 0.0005), 120 (P < 0.0002) and 240 min (P = 0.063; Table 1). In the HAB group, only at 60 min was the peritoneal volume significantly greater during DS 2 vs DS 1 (P < 0.005; Table 1).

The amount of glucose absorbed over the time of the experiment did not differ between the tested solutions. However, treatment of animals with heparin induced a tendency for an increased amount of intraperitoneal dialysate glucose with time on peritoneal dialysis (DS 2 vs DS 1). There was no significant difference in the peritoneal fluid absorption rate (estimated as KE, RISA elimination rate) among the groups, but there was a tendency to higher KE in the AB group (DS 1, AB 0.035 ± 0.008 ml/min vs HAB 0.030 ± 0.004 ml/min, P = 0.156; DS 2, AB 0.040 ± 0.006 ml/min vs HAB 0.034 ± 0.006 ml/min, P = 0.133). During time on

Table 1. The peritoneal dialysate volume (ml) during dwell studies

<table>
<thead>
<tr>
<th>Dwell study</th>
<th>Group</th>
<th>0 min</th>
<th>2 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS 1</td>
<td>AB</td>
<td>30.0 ± 0.0</td>
<td>30.0 ± 0.5</td>
<td>35.0 ± 1.6</td>
<td>37.7 ± 1.6</td>
<td>38.6 ± 1.4</td>
<td>37.0 ± 2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &lt; 0.001 vs HAB</td>
<td></td>
<td>HAB DS 1</td>
<td>HAB DS 1</td>
<td>HAB DS 1</td>
<td>HAB DS 1</td>
</tr>
<tr>
<td></td>
<td>HAB</td>
<td>30.0 ± 0.0</td>
<td>31.1 ± 0.7</td>
<td>39.0 ± 1.2</td>
<td>41.3 ± 1.1</td>
<td>42.0 ± 2.8</td>
<td>38.1 ± 3.5</td>
</tr>
<tr>
<td>DS 2</td>
<td>AB</td>
<td>30.0 ± 0.0</td>
<td>30.1 ± 0.7</td>
<td>39.4 ± 1.3</td>
<td>41.1 ± 2.0</td>
<td>42.3 ± 1.6</td>
<td>38.4 ± 2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &lt; 0.0001 vs HAB</td>
<td></td>
<td>AB DS 1</td>
<td>AB DS 1</td>
<td>AB DS 1</td>
<td>AB DS 1</td>
</tr>
<tr>
<td></td>
<td>HAB</td>
<td>30.0 ± 0.0</td>
<td>30.0 ± 0.8</td>
<td>39.9 ± 1.4</td>
<td>42.9 ± 1.7</td>
<td>43.6 ± 2.4</td>
<td>39.1 ± 2.0</td>
</tr>
</tbody>
</table>
peritoneal dialysis, the peritoneal fluid absorption rate increased in the AB group (DS 1 vs DS 2, \( P < 0.05 \)).

**Discussion**

In this study, rats treated with dialysis solutions containing heparin had significantly increased peritoneal dialysate volumes during DS 1 compared with rats dialysed without intraperitoneal heparin infusion. In addition, at the same time, we observed a tendency for a lower peritoneal fluid absorption rate in the HAB group as compared with the AB group, and the increase in net ultrafiltration in the HAB group can probably be explained by this potential decrease in fluid reabsorption. The higher peritoneal volume in animals exposed to heparin could not be explained by slower absorption of glucose from the dialysate because the amount of the dialysate glucose was similar in both groups. In a previous study, heparin also suppressed peritoneal net fluid absorption, resulting in an increase of ultrafiltration [12]. The following mechanisms have been proposed to explain the favourable effects of heparin on the peritoneal membrane: modulation of synthesis and composition of the extracellular matrix [13,14], and improvement of the anionic charge barrier of the peritoneal membrane [15].

During the present study, we observed two antagonistic effects in the AB group: (i) healing of the peritonaeum after catheter implantation and omentectomy, resulting in increased volume of dialysate; and (ii) damage to the membrane due to its chronic exposure to peritoneal dialysis solution, resulting in significantly higher peritoneal fluid absorption rates in all the dialysed rats at DS 2 as compared with DS 1. These findings are in agreement with previous observations [7]. The anti-inflammatory action of heparin in the HAB group may account for its protective effect against peritoneal damage. In addition, heparin accelerated the healing of acetic acid-induced gastric ulcers in rats in a dose- and time-dependent manner [16], and such an effect might also affect the peritoneal healing.

In summary, the present study shows that heparin may improve peritoneal fluid transport possibly due to better healing and reduced peritoneal inflammation (induced by omentectomy and implantation of the dialysis catheter). We used for the first time an animal model of chronic peritoneal dialysis with repeated dwell studies (with RISA as macromolecular volume marker) and conclude that this novel research tool may improve the understanding of changes in peritoneal transport during the course of the treatment.

**References**