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Effect of fluid supplementation and modality on peritoneal permeability characteristics in a rat peritoneal dialysis model

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submitted
Abstract
Haemoconcentration may influence peritoneal permeability parameters in anesthetized animals without fluid supplementation. Therefore the aim of this study was to investigate the effects of fluid supplementation on peritoneal permeability in an acute peritoneal dialysis model in anesthetized rats.

To study the effect of fluid supplementation on peritoneal permeability characteristics 24 anesthetized male wistar rats were investigated in 3 groups during a 4 hours standardized peritoneal permeability analysis with a 3.86% glucose dialysis solution (SPARa). The groups included a control group with no fluid supplementation (none) (n=8, 372±20.1 g), a group with continuous subcutaneous infusion (sc) of 0.9% NaCl 3 mL/hour (n=9, 364±35.5 g) and a group with continuous intravenous infusion (iv) of 0.9% NaCl 3 mL/hour (n=7, 386±25.7 g). Inflow, sampling and outflow of the dialysate during the SPARa occurred via a canula inserted ip in the lower left quadrant of the abdomen. Blood was drawn at the end of the dwell.

Urine production during the dwell was not different among the groups: none 10.6±5.3mL, sc: 8.0±6.0mL and iv: 10.5±5.6mL. The transcapillary ultrafiltration after 4 hours was significantly higher in the iv group (p<0.05) compared to the other two groups. The net ultrafiltration and the effective lymphatic absorption were similar in all groups. Mass transfer area coefficient of urea in the iv group was significantly greater (155±23.2 µL/min, p<0.003), but not different between the none (118±16.2 µL/min) and sc (123±25.9 µL/min) groups. The glucose absorption, albumin and IgG clearances and the sieving of sodium were alike in all groups.

It can be concluded that only iv fluid supplementation is effective to prevent dehydration and enhances some peritoneal permeability characteristics in anesthetized rats during a 4 hour investigation. It is therefore important to standardize the fluid supplementation.

Introduction
Clinically relevant and standardized experimental models for in vivo studies of specific aspects of peritoneal dialysis are required for further understanding of peritoneal physiology, pathology and their implications for peritoneal dialysis in patients. Several experimental models for the investigation of peritoneal transport characteristics have been reported both in conscious [1-3] and anesthetized [4-6] animals. In some studies the conscious animals had free access to water [2], anesthetized animals were supplemented with fluid during the experiment either intravenously [5] or subcutaneously [6]. The magnitude of the fluid supplementation and the modality of administration have been described, but were not discussed. So far, the effect of haemoconcentration on peritoneal permeability characteristics in anesthetized animals with normal renal function without fluid supplementation during peritoneal dialysis, has not been evaluated.

Therefore, the aim of this study was to investigate the effects of fluid supplementation on peritoneal permeability in an acute peritoneal dialysis
model in anesthetized rats. The peritoneal transport parameters were studied without normal saline supplementation during a 4-hour dwell and with supplementation either subcutaneously or intravenously.

**Methods**

**Animals**

Twenty four male wistar rats (Harlan CBP, Zeist, The Netherlands) were randomly divided into 3 groups. The group without fluid supplementation (none group) during the standardized peritoneal permeability analysis adapted for rats (SPARa), contained 8 rats. Eight were placed in the group with continuous subcutaneous infusion of 3 mL 0.9% NaCl /hour during the SPARa (sc group). Every 30 minutes the sc infusion needle was shifted to an another location on the flanks or back of the rat. Eight rats were investigated during continuous intravenous infusion of 3 mL 0.9 % NaCl/hour in a tail blood vessel (iv group). One rat was shifted from the iv to the sc supplemented group 30 minutes after the start of the SPARa because of a malfunctioning iv needle. Consequently, the final sc group contained 9 rats and the iv group 7. The normal saline solution was preheated to 37°C for both supplemented groups. All rats were anesthetized by intra muscular administration in a hind leg with a mixture of ketamine, xylazine and atropine (8 mg, 4 mg, 5μg per 100 g body weight). The animals were placed on a 37°C heating pad during the SPARa.

**Standard peritoneal permeability analysis in the rat: SPARa**

The SPARa is a modification of the human standard peritoneal permeability analysis (SPA) described by Pannekeet et al [7]. The SPARa is performed during a 4-hour dwell with 30 mL 3.86% glucose based dialysis solution, preheated to 37°C. Dextran 70, 5 g/L (Hyskon®, Medisan Pharmaceuticals AB, Uppsala, Sweden), is added to the test solution as a volume marker for the calculation of fluid kinetics [8]. The procedure includes a rinsing step of the peritoneal cavity with 30 mL 3.86% glucose dialysis solution prior to the test to avoid possible effects of a residual volume present before the onset of the analysis. Directly after drainage of the 4-hour effluent, another rinsing step is performed with 20 mL 1.36% glucose based dialysis solution, to calculate the residual volume in the peritoneal cavity. Both rinsing solutions are preheated to 37°C. Dialysate samples are taken before instillation of the test solution and 10, 30, 60, 120, 180 and 240 minutes after completion of inflow. Inflow, sampling and outflow of the dialysate during the SPARa occurs via an intravenous infusion needle with a pvc-sheet, which is inserted intra-peritoneal, lateral in the left lower quadrant of the abdomen. One mL is obtained for each intermediate sample using a 1 mL syringe which is flushed 3 times to avoid dead space effect. Outflow at the end of the dwell and after both rinsing steps is accomplished through this canula by gravity. Blood is obtained by heart puncture at the end of the experiment; the rats are sacrificed thereafter. Preweighed gauzes are used to absorb the urine during the dwell and weighed thereafter to estimate the urine production. The animals are anesthetized during the whole SPARa procedure. The protocol was
approved by the Committee of Animal Experiments of the University of Amsterdam.

Assays
Total dextran 70 was measured in all dialysate samples by high performance liquid chromatography [9]. Both in plasma and effluent, urea (Hitachi H747, Boehringer Mannheim, Mannheim, Germany) was measured with an enzymatic method. The glucose concentration was assessed by a glucose oxidase-peroxidase assay (SMA II, Technicon, Terrytown, NY, USA). Sodium was measured by an ion selective electrode (Hitachi H747, Boehringer Mannheim, Mannheim, Germany). Total protein in plasma was determined by biureet methodology (Roche, Almere, The Netherlands) using an automated analyzer (Hitachi 747, Boehringer Mannheim, Mannheim, Germany). Plasma osmolality was assessed by a freeze point reduction method using an automated analyzer (EBMC, Kerkdriel, The Netherlands). Albumin concentrations were determined by the bromocresol green method. IgG was measured with a peroxidase sandwich enzyme-linked immuno assay (ELISA). ELISA plates (Maxisorp immunoplate, NUNC, Roskilde, Denmark) were coated with goat anti-rat IgG (Nordic Immunology, Tilburg, The Netherlands). Horseradish peroxidase labeled goat anti-rat IgG (H+L) (Nordic Immunology, Tilburg, The Netherlands) was applied as conjugate and o-phenylenediamine dihydrochloride (Sigma, St Louis, MO, USA) as substrate. Absorbance was read at 490 nm against a buffer blank, and ion exchange and gelfiltration chromatography purified rat IgG (Sigma Aldrich Chemie BV, Zwijndrecht, The Netherlands) was used as a standard.

Calculations
Peritoneal fluid and solute kinetics were calculated as described previously [7,8]. In brief, the transcapillary ultrafiltration increases the intraperitoneal volume, and fluid loss from the peritoneal cavity is assumed to occur by transcapillary back filtration and uptake into the lymphatic system. The resultant of these is the net ultrafiltration. The transcapillary ultrafiltration was calculated from the dilution of the volume marker, dextran 70. The convective disappearance of the volume marker from the peritoneal cavity can be used as an indirect method to estimate the contribution of the peritoneal lymphatics in the absorption of fluid from the peritoneal cavity [10]. These calculations of the effective lymphatic absorption include all pathways of uptake into the lymphatic system, both interstitial and subdiaphragmatic. The change in intraperitoneal volume during the dwell can be calculated from the dilution of the volume marker after correction for incomplete recovery. The net ultrafiltration is the difference between the transcapillary ultrafiltration and the effective lymphatic absorption.

The MTAC of urea was calculated according to the model of Waniek et al. [11] which corrects for convective transport with the application of a correction factor F=0.5, and the solute concentration was expressed per volume of plasma water [12]. The following equation was applied:
In which $V_{10}$ (mL) represents the intraperitoneal volume, and $D_{10}$ (mmol/L) the dialysate concentration of the solute at t=10 minutes, $V$, and $D$ represent these parameters at t=240 minutes. $P$ is the plasma concentration at the end of the dwell. $V$ is the mean intraperitoneal volume calculated as the area under the intraperitoneal volume versus time curve, divided by the dwell time. The area under the curve is determined with the application of the trapezium rule [13].

Glucose absorption was estimated as the difference between the instilled and the recovered amount of glucose, relative to the instilled quantity of glucose. The peritoneal clearances of albumin and IgG were determined according to the equation: $Cl(\mu L/min) = (D-V)/(P-t)$, in which $Cl$ represents the clearance, $D$ the dialysate concentration at the end of the dwell, $V$ is the dialysate volume at the end of the dwell, $P$ is the plasma concentration and $t$ is the duration of the dwell. The size selectivity or intrinsic permeability of the peritoneum was expressed as the IgG/albumin clearance ratio. Aquaporin-mediated water transport was estimated by the sieving of sodium, expressed as the lowest $D/P$ ratio of sodium during the dwell [14,15].

Statistical analysis
Means and standard deviations are presented unless stated otherwise. One way analysis of variance (ANOVA) was performed to compare the results of the three investigated groups. When ANOVA showed a significant difference among the groups, a modified t-test with a Bonferroni correction for multiple comparisons was used to investigate the differences between the groups.

Results
The clinical data and the laboratory investigations are presented in Table 1. Urine production was not affected by the presence or absence of fluid supplementation and/or its route of administration during the SPARa. The concentrations of urea, glucose and total protein in plasma were significantly lower in the group with iv saline administration compared to the other two groups, but that of Na$^+$ was higher (Table 1). Nevertheless, the plasma osmolality was lowest in the iv group. The time courses of the transcapillary ultrasound, effective lymphatic absorption and the resulting change in intraperitoneal volume were determined in each animal. The fluid profiles of each group are shown in Figure 1. The transcapillary ultrasound after 4 hours was significantly greater in the iv group ($p<0.05$), but was not different between the other two groups. The net ultrasound and the effective lymphatic absorption were similar in all three groups. The sieving of sodium, used as an estimate of aquaporin mediated water transport showed no difference among the three groups (Table 2). Parameters of peritoneal solute permeability are summarized in Table 2. Figure 2 shows box and whisker plots of the MTAC of
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urea and the glucose absorption obtained in all three groups. A significantly greater MTAC of urea was found in the iv group (p<0.003) compared with the other groups. The glucose absorption was similar in all groups. Also the clearances of the macromolecules albumin and IgG were alike. The size selectivity to macromolecules, assessed as the albumin/IgG ratio, was not different.

Discussion

An acute peritoneal dialysis model in the rat with normal kidney function was used to investigate the effect of fluid supplementation and its modality on peritoneal permeability characteristics during a 4-hour standardized peritoneal permeability analysis. The magnitude of the continuous supplementation of normal saline used in this study, administered either intravenously or subcutaneously, was based on the volume of net ultrafiltration (9.3±2.0 mL/4-hour) determined during SPARAs performed under similar conditions without fluid supplementation (unpublished). We only found significant differences in the peritoneal permeability characteristics after iv fluid supplementation in comparison with the not supplemented rats and the animals with sc fluid administration. Therefore, the reported 1-2 mL normal saline/hour administrated subcutaneously [6,16] or intravenously [5] might be insufficient for prevention of haemoconcentration or hypovolemia during a 4-hour dwell in anesthetized rats.

We found a significantly higher TCUF after 4 hours in the iv group, which was not different between the other two groups. The greater TCUF at the end of the dwell in the iv group can be explained by the effect of haemoconcentration in the other two groups, possibly due to a change in the transcapillary pressure gradient. This is composed of the crystalloid pressure gradient and the hydrostatic pressure gradient minus the colloid osmotic pressure gradient [17]. The hydrostatic pressure gradient is assumed to be stable during a 4-hour dwell in conscious patients [18]. It is likely that in anesthetized rats independent from fluid supplementation, this gradient also remains stable during the dwell because regulation of the blood flow by precapillary sphincters in the splanchnic circulation. Comparing the iv group with animals without supplementation showed that the mean difference in plasma osmolality between the two groups was 14 mosmol/ kg H2O. The difference was 7 mosmol/L when the osmolarity was calculated from the Na+, glucose and urea concentrations. One milli-osmol exerts an osmotic pressure of 19.3 mm Hg in case the reflection coefficient of a solute to a membrane equals 1.0. assuming an overall reflection coefficient for low molecular weight solutes of 0.03 [19] the difference in the plasma crystalloid osmotic pressure between the two groups would be either be 8 or 4 mm Hg. This implies that the crystalloid osmotic pressure gradient across the peritoneum is 4 to 8 mm Hg higher in the iv group. The colloid osmotic pressure gradient can be estimated from the plasma albumin concentration applying the equation: 0.38 (Palbumin ) + 7.72 [17].
Table 2. Solute permeability parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No Supplementation</th>
<th>Supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose (mg/dL)</td>
<td>72 + 45</td>
<td>72 + 45</td>
</tr>
<tr>
<td>Total protein (mg/dL)</td>
<td>6.2 + 3.4</td>
<td>6.2 + 3.4</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>33 + 4</td>
<td>33 + 4</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.6 + 0.2</td>
<td>1.6 + 0.2</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>1.5 + 0.4</td>
<td>1.5 + 0.4</td>
</tr>
<tr>
<td>Electrolytes (mEq/L)</td>
<td>138 + 2.1</td>
<td>138 + 2.1</td>
</tr>
</tbody>
</table>

Table 1. Clinical data and laboratory investigations.

- Groups: p > 0.003
- Data are expressed as means ± SD
- M: Total protein was significantly lower (p > 0.01) in non-supplemented mice compared to those supplemented
- F: Plasma concentration was different among the groups
- ns: No difference between groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.2 + 0.1</td>
<td>0.5 + 0.1</td>
<td>0.4 + 0.2</td>
</tr>
<tr>
<td>Glucose absorption (%)</td>
<td>80 + 5</td>
<td>90 + 7</td>
<td>85 + 6</td>
</tr>
<tr>
<td>MTAC clearance (L/m²)</td>
<td>10 + 0</td>
<td>12 + 4</td>
<td>10 + 3</td>
</tr>
<tr>
<td>Max D/P Na (mM/mM)</td>
<td>5 + 3</td>
<td>6 + 4</td>
<td>5 + 3</td>
</tr>
</tbody>
</table>

The differences are statistically significant between the groups (p < 0.05).
Figure 1. The time courses of the transcapillary ultrafiltration (●), effective lymphatic absorption(▲) and the resulting change in intraperitoneal volume (■) in the none (upper), sc (middle) and iv group (lower panel). *p<0.05. Means and standard deviations are presented.
This results in a 1.5 mm Hg higher colloid osmotic pressure gradient in the unsupplemented rats, promoting more back filtration in these animals compared to the iv group. The total osmotic pressure gradient across the peritoneum is the sum of the crystalloid and the osmotic pressure gradients. The total osmotic pressure gradient, inducing the transcapillary filtration of fluid from the circulation to the peritoneal cavity will therefore have been 5 to 10 mm Hg higher in the iv group than in the rats without supplementation. This is the most likely explanation for the greater transcapillary ultrafiltration found in the iv group. It was not reflected by a difference in the net ultrafiltration, because this parameter is also dependent on the effective lymphatic absorption [10,20].

The higher plasma concentrations of solutes and the higher osmolality in the group without fluid supplementation in comparison with the other groups suggest that these parameters, obtained at the end of the dwell of the SPARa, will have been lower in the beginning of the investigation. The end value therefore most likely overestimates the average plasma concentration. This could explain the lower MTACurea found in the not supplemented animals compared to the iv rats, in which the end plasma concentration is more likely to represent a steady state. The basis of the MTAC calculation is the \( \frac{(P-D_t)_{10}}{(P-D_t)} \) ratio. The effect of an overestimation of \( P \) is especially important in the denominator, because of the small differences between \( P \) and \( D_t \). An overestimation of \( P-D_t \) will result in a smaller ratio and therefore in a lower MTAC. This is the most probable explanation for the higher MTAC urea in the iv supplemented rats. The finding that the glucose absorption was not different among the three groups suggests that dehydration or fluid supplementation had no effect on the peritoneal vascular surface area. The similar values for peritoneal size-selectivity also argue against an effect of dehydration on peritoneal permeability.
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It can be concluded that the effects of fluid supplementation during peritoneal permeability studies in anesthetized rats are generally minimal. When it is used 3 mL/hour normal saline should be administered intravenously. Subcutaneous supplementation of the same quantity is easier to apply, but insufficient. It is however most important that the conditions of the experiments are standardized, also in regard to the use and route of the fluid supplementation.

References