Experimental and clinical studies on peritoneal physiology and morphology during chronic peritoneal dialysis
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Amphotericin B, mercury chloride and peritoneal transport in rabbits

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submitted
Amphotericin B, HgCl₂ and peritoneal transport in rabbits

Abstract
The effect of glucose induced ultrafiltration in peritoneal dialysis is dependent on the presence and function of ultra small transendothelial cell water channels. The mercury sensitive aquaporin-1 is considered to represent these transcellular pores. Amphotericin B has been reported to increase ultrafiltration in both experimental and patient studies. The objective of this study was to investigate the hypothesis that intraperitoneal amphotericin B increases and mercury chloride inhibits aquaporin-1 mediated water transport in a chronic peritoneal dialysis model in the rabbit.

Eighteen female New Zealand White rabbits were included for peritoneal catheter implantation. Peritoneal transport parameters were determined in all rabbits by standard peritoneal permeability analysis (SPAR) with 3.86% glucose based dialysis solution during a 1-hour dwell prior the intervention SPARs, as a control. Amphotericin B (0.06 mg/kg body weight) was added to the dialysate for 3 (n=9) or 5 consecutive days (n=5) before investigation. Four rabbits were investigated after 3 days intraperitoneal administration (ip) 0.6 mg/kg body weight amphotericin B. In 3 rabbits 0.06 mg/kg body weight liposomal amphotericin B was administered ip during 3 days before intervention SPAR. Fourteen rabbits were investigated during a 1-hour dwell with 0.1mM HgCl₂ containing 3.86% glucose based dialysis solution, while they were anesthetized. Three of these underwent In vivo fixation with glutaraldehyde prior to the HgCl₂ SPAR to prevent toxic effects of mercury on peritoneal tissues.

Intraperitoneal administration of amphotericin B enhanced the change in intraperitoneal volume during a 1 hour dwell after 3 days ip treatment with the low dose (p<0.02), but it did not affect peritoneal solute permeability. The effect on the ultrafiltration was likely mediated by transcellular water channels, but not by aquaporin-1. No beneficial effects on the ultrafiltration were found with prolonged treatment or with the higher dose. Ultrafiltration decreased (8 mL/hour to 1 mL/hour, p<0.03) after ip administration of HgCl₂ with and without in vivo fixation, accompanied by a significant decrease in aquaporin mediated water transport, estimated as the sieving of sodium (p<0.001). Marked increases in the clearances of macromolecules were found after ip HgCl₂ administration due to toxic effects: total protein clearance increased from 97 to 172 µl/min, p<0.005, and albumin clearance from 59 to 158 µl/min, p<0.005. These changes were less pronounced after in vivo fixation.

In conclusion, amphotericin B has likely no clinical relevance in treatment of ultrafiltration failure in PD patients. Aquaporin mediated water transport could be inhibited and consequently ultrafiltration was reduced by ip administration of mercury chloride in our rabbit model.

Introduction
The effectiveness of glucose as osmotic agent in peritoneal dialysis is dependent on the presence and function of ultra small pores in the vascular wall of the peritoneal microvessels [1,2]. The water channel aquaporin-1, which is the most important water channel in the cell membrane of red blood cells and in the proximal ruble [3] is also present in endothelial cells of peritoneal venules and capillaries [4,5]. Its function is dependent on the presence of a crystalloid osmotic pressure gradient. The function of aquaporin-1 can be inhibited by mercurial compounds [6]. Carlsson
et al. described *in vivo* inhibition of transendothelial water transport after intra peritoneal administration of mercury chloride during acute peritoneal dialysis in the rat [7]. Reduction of the transperitoneal water flow and almost complete inhibition of the transendothelial water transport, estimated by the sieving of sodium, was reported. This supports the hypothesis that aquaporin-1 represents the ultrasmall pore system and is involved in glucose induced ultrafiltration during peritoneal dialysis.

Amphotericin B has been shown to increase the water, but not the electrolyte permeability of thin lipid membranes formed *in vitro* from sheep red blood cells dissolved in decane [8]. These findings suggest enhanced expression or function of aquaporin-1 in the erythrocyte cell membrane. This could explain the observations of Maher et al. [9,10] describing increased ultrafiltration after intraperitoneal administration of a high dose of amphotericin B during short dwell exchanges in rabbits. We found similar results in three PD patients treated for fungal infections with amphotericin B intraperitoneally in a therapeutic dose [11]. The greater net ultrafiltration was caused by increased transcapillary ultrafiltration.

The objective of the present study was to investigate the hypothesis that intraperitoneal amphotericin B increases and intraperitoneal mercury chloride inhibits aquaporin-1 mediated water transport in a chronic peritoneal dialysis model in the rabbit. Therefore, amphotericin B was added to the dialysate for 3 or 5 consecutive days previous to the investigation. The inhibitory effect of ip HgCl₂ on aquaporin mediated water transport was studied in anesthetized rabbits. Standardised peritoneal permeability analyses were performed to investigate the effects of these interventions on the peritoneal solute and fluid transport.

**Material and methods**

An adult coil silastic catheter (Coil-Cath™, Accurate Surgical Instruments Corporation, Toronto Canada) was implanted in eighteen non-uremic, non-omentectomized female New Zealand White rabbits (Broekman Institute, Zomeren, The Netherlands). Subsequent to implantation the catheter was flushed daily for 7 days with 2.5 mL heparin solution (5 IU/mL in 0.9% NaCl). Thereafter peritoneal dialysis was performed once a day with a commercially available 1.36% glucose containing dialysis solution (Dianealet™, Baxter Healthcare S.A., Ireland). A rapid exchange of 40 mL/kg body weight, preheated to 37°C, was followed by instillation of the same volume minus the residual volume of the exchange. The instilled dialysate was left to be absorbed overnight. After at least one week of peritoneal dialysis, the peritoneal permeability was investigated by means of a standard peritoneal permeability analysis (SPAR) [12]. The SPAR is a modification of the human standard peritoneal permeability analysis [13]. It uses 1-hour dwells of 40 mL/kg body weight 3.86% glucose dialysate. Dextran 70, 1 g/L, (Macrodex®, NPBI, Emmer-Compascuum, The Netherlands or Hyskon® Medisan Pharmaceuticals AB, Uppsala, Sweden) was added to the test solution to allow calculation of the fluid kinetics [14]. Median body weight on the control SPAR day was 2670 g (2364-3159 g).

Group 1 consisted of 9 unanesthetized rabbits in which a pre-intervention SPAR (control) was performed. These 9 animals were also investigated after 3 days intraperitoneal administration of amphotericin B, 0.06 mg/kg body weight
Amphotericin B, HgCl₂ and peritoneal transport in rabbits

(ampotericin B dissolved in sodium desoxycholate: Fungizone™ Bristol-Myers Squibb BV, Woerden, The Netherlands), added to the dialysate. On the third day a SPAR was performed with the same dose amphotericin B. Five of these nine rabbits were also investigated after 5 consecutive days of i.p. administration of amphotericin B. Group 2 contained four rabbits in which the control SPARs were compared with the data obtained after 3 days i.p. administration of 0.6 mg/kg amphotericin B. Group 3 consisted of 3 rabbits in which paired observations of a control SPAR and a SPAR after i.p. treatment with 0.06 mg/kg liposomal amphotericin B (AmBisome®, Vestar Benelux BV, Odiliënburg, The Netherlands) for 3 days were assessed. Fourteen rabbits of the whole group were anesthetized with a mix of 5 mg/kg xylazine and 35 mg/kg ketamine i.m., for a SPAR with 0.1 mM HgCl₂ containing 3.86% glucose dialysate. In three of these anesthetized animals an in vivo fixation was performed with 50 mL 1% glutaraldehyde (Sigma Chemicals Co., St. Louis, MO) diluted in a phosphate buffer pH 7.4 during 1.5 min [7], directly preceding the HgCl₂ SPAR. The rabbits included for the SPAR with the mercurial compound were sacrificed immediately thereafter. All rabbits underwent a control SPAR before the intervention experiments were carried out. The protocol was approved by the Committee of Animal Ethics of the University of Amsterdam.

Laboratory methods
Sodium concentrations were assessed by an ion selective electrode on a automated analyzer (Hitachi H747, Boehringer Mannheim, Germany). Urea was also determined on this analyzer, using an enzymatic method. Creatinine was measured with an enzymatic PAP⁺ assay on another automated analyzer (Hitachi H911, Boehringer Mannheim, Germany). The glucose concentration was assessed by glucose oxidase-peroxidase assay (SMA II, Thechnicon, Terrytown, USA). Enzymatic methods for the measurement creatinine are influenced by the high glucose concentrations in dialysates [15,16]. For the method used in our laboratory, the following correction factor (cF) was calculated: cF = 3.10^{-}[gluc]² + 0.11[gluc] + 105, in which [gluc] is the glucose concentration of the dialysate. Total protein concentration was assessed in plasma and dialysate samples of the mercury chloride SPARas with or without prefixation and the paired controls by spectrophotometry on an automated analyzer (Hitachi H747, Boehringer Mannheim, Germany). Albumin was determined with the broom cresol green (BCG) method. IgG was measured with a peroxidase sandwich enzyme-linked immuno sorbent assay. ELISA plates (Maxisorp immunoplate, NUNC, Roskilde, Denmark) were coated with IgG goat anti-rabbit/7S antibody (Nordic Immunology, Tilburg, The Netherlands). Horseradish peroxidase labeled goat anti-rabbit IgG (H+L) was used as conjugate (Nordic Immunology, Tilburg, The Netherlands) and o-phenylenediamine dihydrochloride (Sigma, St Louis, MO, USA) as substrate. The reaction was stopped by the addition of 2M H₂SO₄ to each well. Absorbance was read at 490 nm against a buffer blanc and chromatographically purified rabbit IgG (Nordic Immunology, Tilburg, The Netherlands) was applied as standard. Total dextran was determined by high performance liquid chromatography [17].

Calculations
Peritoneal solute transport parameters and fluid kinetics were calculated as has been described previously [12-14]. In brief, the transport of the low molecular weight
solute urea and creatinine, was expressed as the mass transfer area coefficients (MTAC) according to Waniiewski et al. [18] with a modification for the 60 minute dwell applied in this rabbit model. The solute concentration was expressed per volume of plasma water [19]. The MTACs were calculated according the following equation:

\[
MTAC(mL/min) = \frac{V}{t} \ln \left( \frac{V_{10}^{0.5}(P-D_{10})}{V_{t}^{0.5}(P-D_{t})} \right)
\]

in which \( P \) (mmol/L) is the plasma concentration of the solute. \( V_{10} \) (mL) represents the intraperitoneal volume at time \( t=10 \) min and \( D_{10} \) (mmol/L) the dialysate concentration at time \( t=10 \) min, whereas \( V_t \) and \( D_t \) are these parameters at the end of the dwell. \( V \) is the mean intraperitoneal volume (mL). This is the volume determined as the area under the intraperitoneal volume versus the time curve, divided by the dwell time. The application of the correction factor 0.5 as the exponent of the intraperitoneal volume at time \( t=10 \) and \( t=60 \) min, corrects for convective transport [18]. The glucose absorption was estimated as the difference between the instilled and the recovered amount of glucose, relative to the amount of glucose instilled. The clearances of albumin and IgG were calculated according to the equation: \( Cl(g/L) = (D-V)/(P-t) \), in which \( Cl \) represents the clearance, \( D \) (mg/L) is the dialysate concentration and \( V \) (mL) the volume at the end of the dwell. \( P \) represents the plasma concentration (g/L) and \( t \) is the dwell time (min).

Fluid transport across the peritoneum during peritoneal dialysis is influenced by opposing mechanisms. The intraperitoneal volume increases by transcapillary ultrafiltration. Fluid loss from the peritoneal cavity is assumed to occur by backfiltration and uptake into the lymphatic system. The difference between these is the net ultrafiltration. The transcapillary ultrafiltration was determined as the dilution of dextran 70. The transcapillary ultrafiltration rate was calculated by dividing the transcapillary ultrafiltration by the dwell time. The convective disappearance of the volume marker from the peritoneal cavity, was determined to assess the effective lymphatic absorption [20]. These calculations include all pathways of uptake into the lymphatic system, both interstitial and subdiaphragmatic. The change in intraperitoneal volume during the dwell could be calculated as the dilution of the volume marker after correction for incomplete recovery. The intraperitoneal volume is calculated as the instilled volume plus the volume obtained from the transcapillary ultrafiltration. The net ultrafiltration rate is defined as the change in intraperitoneal volume divided by the dwell time. Aquaporin-mediated water transport was estimated by the sieving of sodium, expressed as the dialysate-over-plasma ratio (D/P) of sodium [21,22]. A diffusion correction was made when the initial dialysate concentration differed more than 5 mmol/L from the plasma concentration [23]. The sodium diffusion correction was performed because the concentration difference causes Na\(^+\) diffusion from the circulation to the dialysate, which leads to underestimation of the actual sodium sieving. The MTAC of creatinine, calculated according to Waniiewski [18,19] was used to compute the magnitude of the Na\(^+\) diffusion from the circulation as has been described previously [23].
Table 1. Parameters of peritoneal permeability obtained in rabbits during 1-hour control dwells using a 3.86% glucose dialysis solution and after i.p. administration of amphotericin B.

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>ampho 3d</th>
<th>ampho 5d</th>
<th>ampho 3dH</th>
<th>amBi 3d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=16</td>
<td>n=9</td>
<td>N=5</td>
<td>n=4</td>
<td>n=3</td>
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<tr>
<td>MTACurea (mL/min)</td>
<td>2.50 (1.47-4.26)</td>
<td>2.38 (1.87-4.51)</td>
<td>2.69 (1.65-3.71)</td>
<td>3.38 (2.01-3.78)</td>
<td>2.74 (2.02-4.59)</td>
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<tr>
<td>MTACr (mL/min)</td>
<td>1.91 (1.21-2.46)</td>
<td>2.26 (1.74-2.70)</td>
<td>2.38 (2.07-3.16)</td>
<td>2.16 (2.06-2.31)</td>
<td>2.19 (0.97-2.58)</td>
</tr>
<tr>
<td>glucose absorption (%)</td>
<td>60 (44-70)</td>
<td>61 (43-71)</td>
<td>63 (51-71)</td>
<td>68 (40-76)</td>
<td>65 (62-69)</td>
</tr>
<tr>
<td>albumine clearance (μL/min)</td>
<td>58 (13-153)</td>
<td>48 (16-109)</td>
<td>74 (23-120)</td>
<td>49 (27-87)</td>
<td>73 (44-74)</td>
</tr>
<tr>
<td>IgG clearance (μL/min)</td>
<td>37 (14-93)</td>
<td>38 (14-134)</td>
<td>37 (18-54)</td>
<td>47 (21-71)</td>
<td>43 (31-73)</td>
</tr>
<tr>
<td>TCUF (mL/hr)</td>
<td>42 (27-87)</td>
<td>53 (34-78)</td>
<td>54 (39-59)</td>
<td>27 (18-39)</td>
<td>31 (31-43)</td>
</tr>
<tr>
<td>ELA (mL/hr)</td>
<td>32 (14-57)</td>
<td>30 (15-61)</td>
<td>36 (31-44)</td>
<td>32 (17-42)</td>
<td>23 (22-32)</td>
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<tr>
<td>NUF (mL/hr)</td>
<td>8 (-7-41)</td>
<td>19 (0-47)</td>
<td>9 (-1-27)</td>
<td>-3 (-20-14)</td>
<td>8 (-3-21)</td>
</tr>
</tbody>
</table>

ampho 3d: after 3 days i.p. administration of amphotericin B (1.5 mg/L); ampho 5d: after 5 days i.p. administration of amphotericin B (1.5mg/L); ampho 3dH: after 3 days i.p. administration of the high dose amphotericin B (15 mg/L); amBi 3d: after 3 days i.p. administration of liposomal amphotericin B (1.5mg/L). MTACurea: mass transfer area coefficient of urea; MTACr: mass transfer area coefficient of creatinine; TCUF: transcapillary ultrafiltration; ELA: effective lymphatic absorption; NUF: net ultrafiltration; medians and ranges are presented, when the interventions were investigated with their paired observations no significant differences were found.
Results are presented as medians and ranges unless stated otherwise, as not all data were symmetrically distributed. Wilcoxon matched pairs rank sum test were used for distribution free testing. Repeated measurement analyses of variance were applied to investigate the differences in the time courses of the transcapillary ultrafiltration, the net-ultrafiltration and the sieving of sodium.

**Results**

Amphotericin B

The parameters of peritoneal permeability obtained in all rabbits during a 1-hour control dwell using a 3.86% glucose dialysis solution are presented in Table 1. The data assessed after i.p. administration of amphotericin B in different concentrations and different treatment durations, are also shown in Table 1. No significant differences in the peritoneal permeability characteristics were found between the control and the intervention experiments based on the paired observations obtained in each rabbit, independent from the concentration of amphotericin B or the number of days the treatment was given. However, amphotericin B administration resulted in a significantly greater change in the intraperitoneal volume (<0.022) during the dwell after 3 days i.p. administration of 1.5 mg/L when all time points were taken into account (Figure 1, left panel). The time course of the transcapillary ultrafiltration was higher when all time points were taken into account, but not significant. This effect was not seen after 5 subsequent days of i.p. administration of amphotericin B in the same concentration (Figure 1, left panel). Moreover, 3 days i.p. treatment with the high dose amphotericin B resulted in a significantly lower transcapillary ultrafiltration (<0.036) accompanied by a lower change in intraperitoneal volume (<0.037) during the dwell. In contrast with the expectation, the effect of amphotericin B on the aquaporin mediated water transport resulted in a decrease of the maximum sodium sieving. Because the sieving of sodium is expressed as a percentage of the initial dialysate-to-plasma ratio, a decrease in aquaporin mediated water transport results in an increase in the percentage: 90.4% during the control SPAR and 93.0% after 3 days i.p. administration of amphotericin B (1.5 mg/L dialysis solution, <0.003), and 90.2% during the control SPAR and 94.2% after 5 days treatment with the same concentration amphotericin B (<0.035). A similar decrease in the sodium sieving was seen after 3 days i.p. administration with the high dose amphotericin B: control value 91.9% and 93.9% after 3 days treatment (<0.01). Three days i.p. administration with liposomal amphotericin B (1.5 mg/L) did not affect the peritoneal permeability characteristics significantly (Table 1, Figure 1, left panel).

*Mercury chloride*

The parameters of peritoneal fluid kinetics, the mass transfer area coefficients (MTACs) of small solutes and the clearances of macromolecules obtained during the control SPAR and after i.p. administration of HgCl₂ with or without in vivo fixation, are presented in Table 2. The MTACs of urea and creatinine, and the glucose absorption did not change significantly after the addition of mercury
Table 2 Parameters of peritoneal permeability obtained in rabbits during 1 hour control dwells using a 3.86% glucose dialysis solution and after the administration of mercury chloride to the test solution.

<table>
<thead>
<tr>
<th></th>
<th>control (n=14)</th>
<th>HgCl₂ (n=11)</th>
<th>HgCl₂ prefix (n=3)</th>
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<tr>
<td>MTACurea (mL/min)</td>
<td>2.57 (1.13-4.26)</td>
<td>2.70 (2.04-7.27)</td>
<td>1.54 (1.13-1.69)</td>
</tr>
<tr>
<td>MTACcr (mL/min)</td>
<td>1.94 (1.24-3.10)</td>
<td>1.87 (1.29-3.30)</td>
<td>0.79 (0.59-0.92)</td>
</tr>
<tr>
<td>glucose absorption (%)</td>
<td>61 (44-70)</td>
<td>55 (32-82)</td>
<td>38 (38-48)</td>
</tr>
<tr>
<td>total protein clearance (μL/min)</td>
<td>97 (66-164)</td>
<td>172 (96-234)*</td>
<td>102 (101-121)</td>
</tr>
<tr>
<td>albumine clearance (μL/min)</td>
<td>59 (13-153)</td>
<td>158 (41-335)*</td>
<td>81 (62-86)</td>
</tr>
<tr>
<td>IgG clearance (μL/min)</td>
<td>43 (19-102)</td>
<td>98 (34-446)</td>
<td>46 (43-75)</td>
</tr>
<tr>
<td>TCUF (mL)</td>
<td>45 (27-87)</td>
<td>39 (25-80)</td>
<td>33 (8-49)</td>
</tr>
<tr>
<td>ELA (mL)</td>
<td>32 (14-57)</td>
<td>41 (24-103)**</td>
<td>27 (23-44)</td>
</tr>
<tr>
<td>NUF (mL)</td>
<td>8 (-7.41)</td>
<td>1 (-64.19)***</td>
<td>5 (-15.6)</td>
</tr>
</tbody>
</table>

MTAC urea : mass transfer area coefficient of urea; MTACcr : mass transfer area coefficient of creatinine; TCUF: transcapillary ultrafiltration; ELA: effective lymphatic absorption; NUF: net ultrafiltration; medians and ranges are presented, the interventions were investigated with their paired controls.*p<0.005, **p=0.023, ***p=0.029.
The change in intra peritoneal volume during the dwell was significantly greater from the control after 3 days i.p. treatment with 1.5 mg/L amphotericin B (p=0.022), after 3 days i.p. administration of the high dose amphotericin B (15 mg/L) it was significantly lower compared to the controls (p=0.037). After 5 days i.p administration of amphotericin B (1.5 mg/L); after 3 days i.p. administration of liposomal amphotericin B (1.5 mg/L). Right panel. The change in the intra peritoneal volume after addition of HgCl₂, after in vivo fixation and i.p. administration of HgCl₂ and the controls. p=0.029: the change in intraperitoneal volume at the end of the dwell was significantly lower after the addition of HgCl₂ compared to the paired controls. Means and SEM are presented.

Intraperitoneal administration of mercury chloride caused an increase in the effective lymphatic absorption (ELA) (p<0.023) accompanied by a decrease in the net ultrafiltration (p<0.029). Also a marked decrease in the sodium sieving was seen after the i.p. administration of mercury chloride, resulting in a significantly different time course of the sodium sieving (Figure 3 left panel, p<0.001) in comparison with the paired controls. The in vivo fixation previous to the administration of HgCl₂, resulted in a similar finding. However, the difference in the time course was not significant compared to the controls (Figure 3 right panel). The sieving of sodium was significantly more blunted at the end of the dwell after i.p. administration of mercury chloride without (94.3 %) than with in vivo fixation (93.2%, p<0.02).
Amphotericin B, HgCl₂ and peritoneal transport in rabbits

Figure 2. The paired data of the clearances of total protein (left panel), albumin (middle panel) and IgG (right panel) after i.p. administration of HgCl₂. The clearances of total protein (p<0.005) and albumin (p=0.005) were significantly higher after i.p. administration of HgCl₂. The clearance of IgG increased less pronounced (p=0.07).

Discussion
The present study describes the effects of intraperitoneal administration of amphotericin B and mercury chloride on peritoneal fluid kinetics and solute permeability, investigated in our chronic peritoneal dialysis model in the rabbit.

Intraperitoneal administration of amphotericin B did not affect peritoneal solute permeability. A significantly greater change in the intraperitoneal volume after 3 days ip treatment with the low dose amphotericin B was found, due to increased transcapillary ultrafiltration. This is in line with the observations of Maher et al [9]. They reported an increase in the ultrafiltration after a single and 10-times higher dose administered intraperitoneally during a short dwell in an acute rabbit PD model. In a later study [24]. In a later study these authors found the increase in ultrafiltration only when amphotericin B was administered as a powder. When its solvent sodium-desoxycholate was administered peritoneally irritation was observed, leading to decreased ultrafiltration and increased solute clearances. In the present study a change in the intraperitoneal volume was found only after 3 days ip treatment with the low dose amphotericin B. This suggests that longer treatment with a higher dose would diminish the higher ultrafiltration due to sterile inflammation. However, no changes were found in solute transport parameters. Wang et al. [25] investigated the effects of a single dose of amphotericin B in an acute model in the rat. They used a high and a low dose similar to those in our experiments. Only for the high dose some effect on ultrafiltration was seen. Also an increase in the D/P and MTAC of potassium was found. This did not occur in our experiments (data not shown). The slight differences between the results of Wang et al and those of the present study are likely to be caused by the use of different animal models and different set-up of the experiments.

Liposomal amphotericin B did not influence any of the peritoneal permeability parameters during 3 day ip treatment, most likely because dissociation of the liposomes has to occur to release amphotericin B in the peritoneal cavity.

Amphotericin B has been described to enhance the water and nonelectrolyte
Aquaporin-11 is a mercury sensitive transcellular water channel [3,7] which could be confirmed in our study. The net ultrafiltration significantly decreased after ip administration of HgCl₂ in combination with an increase in the lymphatic absorption. Furthermore, a significant decrease in aquaporin mediated water transport was seen, estimated by the sieving of sodium. Time limited prefixation with glutaraldehyde was reported to not adversely affect aquaporin mediated water transport in the kidney [28] and in the peritoneum of a rat [7]. This was confirmed in the present study, estimated by the sieving of sodium which was similar to the observations found without the prefixation. In vivo fixation prior to the ip mercury chloride administration was performed to reduce the tissue damage and a peritonitis-like reaction caused by HgCl₂. Glutaraldehyde fixation has been reported to reduce the vascular peritoneal surface area by about 50% [7]. This is in line with the lower MTACs of urea and creatinine, and the decrease in the glucose found in the present study. The clearances of macromolecules increased markedly after the i.p addition of mercury chloride without in vivo fixation probably due to the toxic effects. The changes in the clearances of macromolecules were less pronounced in the animals with in vivo fixation prior to the i.p. administration of mercury chloride.

Figure 3. Aquaporin mediated water transport assessed as the sieving of sodium after i.p. administration of HgCl₂ without in vivo fixation (▲ left panel), and with in vivo fixation (● right panel) and their controls ○. Sieving of sodium during the dwell was blunted after both interventions (p<0.001). Medians and ranges are presented.
Amphotericin B, HgCl₂ and peritoneal transport in rabbits

The *in vivo* glutaraldehyde prefixation has been described as 'gentle' in anesthetized animals [28]. We found this not to be the case and therefore limited the study to 3 investigations.

In summary, our results show that intraperitoneal administration of amphotericin B enhanced the ultrafiltration during a 1-hour dwell after 3 day ip treatment with the low dose. Prolonged treatment, or treatment with a higher dose was not beneficial. This effect was likely to be mediated by enhanced transcellular water transport, but not by aquaporin-1. Therefore, amphotericin B is likely to have no clinical relevance with respect to treatment of ultrafiltration failure in PD patients. Furthermore, aquaporin mediated water transport was successfully inhibited by intraperitoneal administration of mercury chloride.

References


