Free water transport, small pore transport and the osmotic pressure gradient

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Abstract

Background. Water transport in peritoneal dialysis (PD) patients occurs through the small pores and water channels, the latter allowing free water transport (FWT). The osmotic gradient is known to be one of the major determinants of water transport. The objective of the study was to analyse the relation between each transport route and the osmotic gradient.

Methods. The 4-h standard peritoneal permeability analyses of 80 stable PD patients were studied. Small pore transport (SPT) was calculated based on the transported amount of sodium. FWT was calculated by subtracting SPT from transcapillary ultrafiltration (TCUF). Water transport rates were determined. The osmotic gradient was calculated. The slope of the relation between FWT rate and osmotic gradient (slopeFWT), and the elimination constant (KsPT) of the exponential relation between SPT rate and osmotic gradient (KsPT) were calculated for every patient.

Results. The FWT rate was related to the osmotic gradient (P = 0.001). A similar correlation was also found between the SPT rate and osmotic gradient when fitted exponentially (P = 0.005). The rates of FWT decreased significantly between each time point during the whole dwell. The SPT rates decreased significantly within the first half of the dwell and levelled off thereafter. No correlations were found between the slopeFWT, KsPT and PD duration. The slopeFWT of the relationship between the FWT and the osmotic gradient is an indirect measurement of the amount of functioning water channels. Similarly, the KsPT value represents the number of functioning small pores. The absence of a relationship of these parameters with the duration of PD suggests opposing mechanisms, for instance a lower number of functioning pores in combination with an increased vascular surface area.

Conclusion. The curves of the relationship between FWR, SPT and OG support the assumption that FWR is much more dependent on the OG than SPT. Non-osmotic determinants are likely to be important in small pore fluid transport.

Keywords: free water transport; osmotic pressure gradient; small pore transport; transcapillary ultrafiltration

Introduction

Ultrafiltration is one of the objectives of peritoneal dialysis (PD) treatment. The fluid flow is induced by the action of Starling forces [1,2]. In the absence of an osmotic agent, the transcapillary hydrostatic pressure gradient during continuous ambulatory peritoneal dialysis (CAPD) favouring ultrafiltration towards the peritoneal cavity is exceeded by the colloid osmotic pressure gradient. This results in fluid reabsorption into the capillaries [3,4]. Therefore, an osmotic agent is needed in the dialysate to prevent uptake of fluid from the peritoneal cavity and produce net ultrafiltration.

According to the three-pore model of peritoneal transport [5,6], water is transported mainly across small and ultrasmall pores. A negligible amount is transferred across the large pores. Small pores with a radius of 40–50 Å are assumed to be present in a large amount in the peritoneal vessels. Their contribution to the total fluid removal is roughly 60% of the total transcapillary ultrafiltration (TCUF) [6]. Aquaporin 1 in peritoneal capillaries and venules has been identified as the anatomic equivalent of the ultrasmall pores [7]. Water channels are considered the main pathway of crystalloid-osmosis-induced water flow. Although the amount of aquaporins is markedly lower than the amount of small pores, the ultrafiltration capacity across these aquaporins is markedly higher than the ultrafiltration capacity of small pores. As a result, water channels are responsible for 40–50% of fluid removal within the first hour of a dwell performed with a 3.86% glucose-based dialysis solution [6,8].

The objective of the present study was to investigate the effect of the osmotic gradient on water transport through small pores and water channels.
Subjects and methods

The study is an extension of a previous analysis on the contribution of free water transport (FWT) and small pore transport (SPT) to the total fluid removal in PD [9]. Standard peritoneal permeability analyses (SPA) of 80 stable PD patients (39% male) were studied. The median age was 57 years (range 21–79). The median duration of PD was 15 months (range 1–73); 8% of the patients were on PD for more than 3 years. The SPA was performed under standardized conditions.

The patients were peritonitis free at and during the 4 weeks preceding the test. All patients used commercially available glucose-based dialysate (Dianeal®®, Physioneal®®, Baxter Healthcare Ltd, IRL-Dublin, Ireland); 47 of them used 7.5% icodextrin (Extraneal®®, Baxter Healthcare Ltd, IRL-Dublin, Ireland) for the long dwell. One patient had one dwell with an amino-acid-based solution (Nutrineal®®, Baxter Healthcare Ltd, IRL-Dublin, Ireland).

Procedure

The SPAs were performed during 4-h dwell periods, as described previously [10]. Briefly after a rinsing procedure, a fresh 3.86% glucose-based dialysis solution (Dianeal®®, Baxter Healthcare Ltd, IRL-Dublin, Ireland) was instilled for a test dwell. Dextran 70 (Hyskon®®, Pharmacia AB, Emmer-Compascuum, Sweden or Macrodex, NPBI, Emmer-Compascuum, The Netherlands), 1 g/L, was added to calculate peritoneal fluid kinetics [11]. The dialysate samples were taken before instillation and at multiple time points during the test: 10, 20, 30, 60, 120, 180 and 240 min after inflow of the test solution. Blood samples were taken at the beginning and at the end of the test. To prevent possible anaphylactic reactions to dextran 70, 20 mL of dextran 1 (Promiten®®, Gynotec, Malden, The Netherlands) was injected intravenously before instillation of the test solution [10].

Measurements

The total dextran concentration in the dialysate was determined by high-performance liquid chromatography [12]. Creatinine and urate in plasma and effluent were measured by enzymatic methods (Hitachi, Boehringer Mannheim, Germany). Sodium was determined with ion-selective electrodes on the Modular P800 chemistry analyser from Roche Diagnostics (GMBH Manheim, Germany), using an indirect method. The glucose concentration was measured by the glucose oxidase–peroxidase assay (SMA II, Technicon, Terrytown, NJ, USA). Albumin was measured by nephelometry (BN 100, Behring, Marburg, Germany) using commercial antisera (Dakopatts, Glostrup, Denmark).

Calculations

Peritoneal fluid transport parameters were calculated in each individual’s SPA as described previously [10]. Solute concentrations are expressed as the solute concentration per volume of plasma water [13].

The changes in the in situ intraperitoneal volume arise from transcapillary ultra- and backfiltration and lymphatic absorption. The changes in the in situ intraperitoneal volume during the dwell were calculated by means of dextran dilution after correction for incomplete recovery [14]. Dextran loss was calculated as the difference between the amount of dextran 70 instilled and the total amount of dextran recovered, that is in the drained test bag, in the residual volume after drainage and in the samples that were taken during the dwell. This convective loss of dextran during the dwell was expressed as peritoneal dextran clearance (dextran loss divided by the geometric mean of the initial and final dextran concentration in the dialysate, that is in the drained dialysate after 4 h) and considered to represent the effective lymphatic absorption [14]. With this methodology, all pathways of uptake into the lymphatic system, subdiaphragmatic and interstitial, are included. The TCUF during the dwell was calculated by subtracting the initial in situ intraperitoneal volume from the theoretical intraperitoneal volume at any time point when both effective lymphatic absorption and dextran loss due to the sampling would not have been present [11].

For the calculation of water transport, a correction for sodium diffusion from the circulation to the dialysate was performed using the mass transfer area coefficient (MTAC) of urate [16]. This correction is based on our previous finding that the mean MTAC\(\text{Na}^{+}\) was equal to that of urate, that is 8.3 mL/min [17]. Also, a good correlation was present between the MTAC\(\text{urate}\) and the MTAC\(\text{Na}^{+}\) (\(P < 0.003\)). As the MTAC is a parameter for diffusive transport, the MTAC\(\text{urate}\) can be used to predict the dialysate/plasma concentration gradient of \(\text{Na}^{+}\) by diffusion, because the initial concentration gradient of sodium is known. The dialysate \(\text{Na}^{+}\) concentration in the absence of diffusion can be calculated at any time point during the dwell by subtracting, from the measured gradient, the estimated concentration gradient of \(\text{Na}^{+}\) by diffusion.

FWT was calculated by subtracting TCUF coupled to Na\(^+\) transport from the total ultrafiltration [6]. The time point 10 min after completion of inflow was taken as the start value. The total ultrafiltration at any time point of the dwell was calculated by subtracting the theoretical intraperitoneal volume at 10 min from the theoretical intraperitoneal volume at that moment.

SPT was calculated by multiplying the theoretical intraperitoneal volume at any time point during the dwell by the dialysate sodium concentration corrected for diffusion. By subtracting the amount of sodium at 10 min from the amount at every point, the quantity of sodium transported within each period of the dwell was calculated. Fluid transport through the small pores at every time point was computed by dividing the amount of transported sodium by the sodium concentration in the small pores, that is the mean of the plasma and the dialysate sodium concentration.

At each moment, the fluid transport through the small pores was subtracted from the total ultrafiltered fluid volume, resulting in FWT within every analysed period of the dwell. Both FWT and small pore fluid transport are expressed as absolute values.
The rate of fluid transport at any time interval was calculated as the fluid volume transported in that time interval divided by the number of minutes.

The crystalloid osmotic pressure gradient across the peritoneal membrane was calculated in each individual's SPA by means of the difference between the plasma and dialysate osmolarity at every time point. For the calculations of osmolarity, plasma and dialysate glucose, urea and sodium concentrations were used. According to Van’t Hoff’s law, every mosmol/kg H₂O exerts an osmotic pressure of 19.3 mmHg in the case of an ideal semipermeable membrane (reflection coefficient 1.0). The crystalloid osmotic pressure gradient is thus the product of the crystalloid osmolarity gradient and the reflection coefficient of the abovementioned low molecular weight solutes, multiplied with 19.3. The reflection coefficient of glucose across the water channels is 1; a value of 0.03 has been calculated across the small pores in CAPD patients [17,18]. A similar value was used for urea and sodium. The plasma colloid osmotic pressure was assessed in each individual’s SPA according to Ho-Dac-Pannekeet et al. [15,18] using the plasma albumin concentration in every patient and a correction for the Gibbs–Donnan equilibrium. The osmotic pressure gradient across small pores and water channels was calculated as the difference between the crystalloid and colloid osmotic gradient at every time point.

The relationship between the crystalloid osmotic pressure gradient at any time point and FWT was expressed in every patient as the slope of the linear relationship between these parameters (slopeFWT). Similarly, the elimination constant was calculated for the crystalloid osmotic pressure gradient and SPT (K_SPT). This was done because an exponential relationship appeared to be present between these two parameters (see the Results section). The slopeFWT of the relationship between FWT and the osmotic gradient can be considered an indirect measurement of the amount of functioning water channels. The K_SPT represents the number of functioning small pores. For the analyses, only the slopes and K_e values that reached statistical significance (R > 0.84, P < 0.05) were used.

### Statistical analyses

Mainly due to the asymmetric distributions, the results are expressed as medians and ranges. In Figure 1, means and standard errors of the mean are given. The Mann–Whitney test was used to compare differences between the different time points during the SPA. Spearman’s and Pearson’s correlation coefficients were applied for the correlations. For the calculation of the slopes and K_e values, linear and exponential regressions were used.

### Results

The changes of the osmotic pressure gradient across the small pores and water channels at different time points during the 4-h dwell are listed in Table 1. The colloid osmotic pressure gradient at the start was 18 mmHg, range 16–25. It was 19 (15–27) mmHg at the end of the dwell. No difference was present between the colloid osmotic pressure gradient at the start and the end (P = 0.8). A significant decrease in every time interval was found for the crystalloid osmotic pressure gradient. In Table 2, the rates of water transport through the small pores and water channel at every time interval are shown. The rates of FWT decreased significantly between each time point during the whole dwell.

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**Table 1.** The osmotic pressure gradients (crystalloid + colloid) across the small pores and water channels during the 4-h dwell. Medians and ranges are given

<table>
<thead>
<tr>
<th>Time points (min)</th>
<th>Small pores</th>
<th>Water channels</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>51 (35 to 71)</td>
<td>3531 (2703 to 4594)</td>
</tr>
<tr>
<td>30</td>
<td>43 (26 to 65)</td>
<td>3146 (2259 to 4285)</td>
</tr>
<tr>
<td>60</td>
<td>31 (16 to 58)</td>
<td>2591 (1719 to 4020)</td>
</tr>
<tr>
<td>120</td>
<td>16 (0 to 34)</td>
<td>1800 (956 to 2810)</td>
</tr>
<tr>
<td>180</td>
<td>7 (−6 to 23)</td>
<td>1369 (627 to 2250)</td>
</tr>
<tr>
<td>240</td>
<td>2 (−28 to 18)</td>
<td>1003 (241 to 1903)</td>
</tr>
</tbody>
</table>
The SPT rates decreased significantly within the first half of the dwell and levelled off after 120 min.

The relationships between the rates of SPT, the rates of FWT and the osmotic pressure gradients across these pores are shown in Figure 1. The rate of FWT calculated on the mean values at every time point decreased gradually with the decline of the gradient ($P = 0.001$). The SPT rate decreased significantly with the loss of gradient up to 16 mmHg ($P = 0.005$) and it remained stable despite a further decrease of the osmotic pressure gradient. The osmotic gradient of 16 mmHg corresponds with the time point 120 min after the start of the dwell.

The median slope of the relationship between the FWT rate and the crystalloid osmotic pressure gradient ($slope_{FWT}$) of all patients included in the study was 0.003 mL/min/mmHg (range 0.001–0.005). The median $K_{SPT}$ of the curve representing the correlation between the rate of water transport across the small pores and the crystalloid osmotic gradient was 0.20 mmHg$^{-1}$ (range 0.01–0.47). No relationships between $slope_{FWT}$, $K_{SPT}$ and duration of PD treatment were found.

### Discussion

Fluid transport in PD occurs primarily across the small pores and water channels [2]. In the present study, the essential role of the crystalloid osmotic gradient for the activity of the water channels has been quantified. However, the crystalloid osmotic pressure gradient is only one of the determinants of SPT.

Glucose is used as an osmotic agent to create a crystalloid osmotic pressure gradient across the peritoneal membrane. The potency of glucose to maintain this gradient is determined by the resistance of the membrane to glucose transport. This is expressed by the osmotic reflection coefficient, which was estimated to be 0.03 for small pores and 1 across water channels [18]. Although the reflection coefficients of urea and Na$^+$ are marginally lower than that of glucose, we used the value of 0.03 for reasons of simplicity. This may be one of the reasons for a slight underestimation of FWT in the last part of the dwell [19].

Crystalloid osmotic pressure gradients across water channels and small pores as calculated in the present study are in accordance with the data described previously [20]. No osmolality measurements were done. One could argue that the calculated osmotic pressure gradient might have been influenced by unmeasured plasma maltose and other icodextrin metabolites that are present in the patients who used icodextrin in their normal dialysis schedule. Data from the MIDAS study show that the mean plasma maltose concentration was 1.2 g/L [21]. Given a molecular weight for maltose of 342 Da, it can be calculated that the contribution of maltose to plasma osmolality in these patients is 3.5 mosmol/L. The higher molecular weight degradation products of icodextrin are likely to contribute about 0.5 mosmol/L. Consequently, the effect of chronic icodextrin administration on the osmolality gradient is unlikely to exceed 4 mosmol/L, that is it may lead to a reduction of the crystalloid osmotic pressure gradient of 2 to 3 mmHg.

No direct measurement of the colloid osmotic pressure gradient was done, but it was estimated using individual values for plasma albumin. The values we found were lower than those assumed in the other studies [2] because the serum albumin concentration was lower in the present study than previously reported [22]. The discrepancy could be explained by the method used for the albumin determination. In most clinic studies a dye-binding method using brom cresol green or brom cresol purple is usually employed for the plasma albumin measurement. It has been shown previously that the dye-binding methods overestimate plasma albumin when compared with immunochromatographic methods [23]. The median plasma albumin concentration measured by nephelometry in our study was 27 g/L. Values reported in 480 prevalent PD patients [22] averaged 36 g/L using dye-binding methods. This is close to the albumin value taken for the calculations of the colloid osmotic pressure in the studies by Rippe et al [2]. However, when the value found in incident patients was recalculated to the plasma albumin level that could be expected when an immunoturbidimetric method would have been used, than a serum albumin concentration of only 29 g/L would have been likely. This value is similar to that found in the present and also other studies measuring plasma albumin concentration using an immunochromatographic method [23].

The lower value of the colloid osmotic pressure gradient in the present study affects the total osmotic pressure gradient favouring water transport towards the peritoneal cavity, also at the end of the 4-h dwell. In our study, the SPT rate stabilized at a level of an osmotic gradient of around 16 mmHg, corresponding to a crystalloid osmotic pressure gradient of ~40 mmHg. Taking into account that the colloid osmotic pressure gradient does not change in the second half of the dwell and the crystalloid osmotic pressure gradient decreases gradually, one could hypothesize that non-osmotic determinants are likely to cause the cessation of the decrease in the SPT rate. The hydrostatic pressure gradient is probably the most important non-osmotic determinant. We used a fixed value for the hydrostatic pressure in peritoneal capillaries of 17 mmHg and an average value of 8 mmHg for the i.p. pressure. It is a weakness of the study that i.p. pressure measurements were not done. On the other hand, we do not know the correctness of the assumed hydrostatic pressure value of 17 mmHg in peritoneal capillaries and its inter- and intraindividual differences.

Our findings are in contrast with the three-pore model simulations [2,18], in which the transperitoneal colloid osmotic pressure gradient is set at 22 mmHg, based on a
In this model, the MTACurate is used to predict the amount of fluid transport across water channels is known to be induced by crystalloid osmosis. Also in the present study, a strong correlation was found between the FWT rate and the crystalloid osmotic pressure gradient. Fluid transport across water channels in the second half of the dwell becomes marginal because of a decrease in the osmotic gradient. The slightly negative values for FWT at the end of the dwell are likely to be an artefact. This may have been caused by correction for sodium sieving for diffusion [16]. In this model, the MTACurate is used to predict the amount of sodium transported by diffusion. In the case of a perfect correction for sodium diffusion the D/P ratio should remain constant after reaching its lowest value. This is not really the case in our model [16]. D/P sodium shows a slight increase in the second half of the dwell, especially between 180 and 240 min. This may lead to an underestimation of SPT and thereby to a minor underestimation of the FWT rate. We think this could explain the slightly negative values, found in some patients during the last hour of the dwell.

The slopes of the curves representing the relationship between water transport rate and the crystalloid osmotic pressure gradient (slopeFWT and Kspt) are dependent on the number of functional pores involved in fluid transport. An increased number of peritoneal vessels associated with an augmentation of the anatomic vascular surface area has been shown in long-term PD patients. Also, impairment in peritoneal FWT has been found [6]. The absence of a relationship between slopeFWT, Kspt and duration of PD as found in our study might therefore be explained by two opposing mechanisms: a lower amount of functional pores on one hand and an increase in the vascular peritoneal surface area on the other.

In all clinical studies including the present one, a lumped model for peritoneal transport during PD is used, that is all tissues involved are considered to behave as a single dialysis membrane. Furthermore, the capillary wall is considered to be the major restriction barrier to transport, and it is assumed that transport occurs through a system consisting of three pores [1,5,8,18]. It should be appreciated that only the ultrasmall has been identified as aquaporin-1 [24]. The other pores have never been identified with certainty. Some recent studies focussed attention on a potential role of the endothelial glycocalyx, an intraluminal layer consisting of glycosaminoglycans and hyaluronan, as an important resistance barrier, especially for the transport of macromolecules [25]. Distributed models of peritoneal transport are aimed to take these properties of various peritoneal tissues into account. However, due to their complexity they cannot be used in clinical studies. Although the lumped models are a simplification by definition, they have been extremely useful for assessment of overall peritoneal transport properties.

It can be concluded that FWT is strongly dependent on the crystalloid osmotic pressure gradient while the osmotic pressure dependence is less pronounced in the small pores. The efficiency of water transport across ultrasmall pores decreases due to a fall in the crystalloid osmotic pressure gradient. Non-osmotic determinants are likely to be important in small pore fluid transport.

Conflict of interest statement. None declared.

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