Removal of middle molecules and protein-bound solutes by peritoneal dialysis and relation with uremic symptoms

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Background. Current guidelines for peritoneal dialysis adequacy are based on kinetics of small water-soluble molecules and do not consider the role of other compounds such as middle molecules and protein-bound solutes. Information on the elimination characteristics of the latter solutes by peritoneal dialysis is limited. Moreover, their relation with uremic symptoms remains unclear. The aim of the present study was (1) to investigate the relative contribution of residual renal function to the overall clearances of β₂-microglobulin (β₂m), a middle molecule, and p-cresol, a protein-bound solute, in adults on peritoneal dialysis as compared to small water-soluble molecules and (2) to evaluate relations between serum levels and uremic symptoms.

Methods. We performed a cross-sectional observational study, including 30 nonanuric peritoneal dialysis patients. Total, peritoneal, and renal clearances were calculated for urea nitrogen, creatinine, phosphate, and 2-microglobulin (β₂m), and p-cresol, a protein-bound solute, in adults on peritoneal dialysis as compared to small water-soluble molecules and 2m. All patients were asked to complete a uremic symptom questionnaire.

Results. Declining total clearances (L/week/1.73 m²) were measured for urea nitrogen, creatinine, phosphate, β₂m, and p-cresol, respectively: 97.3 ± 4.6, 98.9 ± 6.1, 64.0 ± 3.4, 23.1 ± 2.6, and 17.5 ± 2.3 (Friedman test P < 0.001). Conversely, the contribution of residual renal function (%) to the respective solute clearances increased significantly: 31.6 ± 3.2, 51.0 ± 4.0, 42.4 ± 4.0, 68.0 ± 5.4, 61.9 ± 4.6 (Friedman test P < 0.001). The serum level of p-cresol, but of none of the other solutes examined, correlated significantly with the symptom score (Pearson r = 0.48, P = 0.008).

Conclusion. During peritoneal dialysis p-cresol behaves like β₂m, probably due to its protein binding. The total clearance of both molecules is significantly lower as compared to water-soluble solutes and mainly depends on renal function. Our data further suggest that protein-bound solutes are involved in the pathophysiology of uremic symptoms.

Key words: peritoneal dialysis, protein-bound solutes, residual renal function, symptoms.

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The aim of this cross-sectional observational study was to investigate the relative contribution of residual renal function to the overall clearances of β2m and p-cresol in adults on peritoneal dialysis as compared to small water-soluble solutes and to evaluate the relation between the serum level of individual retention solutes and the presence of well-recognized uremic symptoms.

METHODS

Patients and study design

We performed a single-center cross-sectional observational study. Thirty nonanuric patients with ESRD treated with peritoneal dialysis at the University Hospital Leuven were included. Fifteen patients were on automated peritoneal dialysis (APD) and 15 were on CAPD. The causes of ESRD were diabetic nephropathy (N = 5), polycystic kidney disease (N = 4), glomerular disease (N = 12), tubulointerstitial disease (N = 4), and unknown etiologies (N = 5). Demographic (age, gender, weight, and length) and clinical data (dialysis duration, medication, and comorbidity) were collected by reviewing the medical records. Comorbidity was scored according to Davies et al [19] and reported as low, medium or high grade. For the determination of clearances of urea nitrogen, creatinine, phosphate, p-cresol, and β2m, a mid-day blood sample was taken and total amounts of urine and peritoneal drainage were collected during the preceding 24-hour period, weighed, and sampled. All samples were stored at −80°C until analysis. For the evaluation of the relation between the serum level of individual solutes and uremic symptoms, the patients were asked to complete a uremic symptom questionnaire. The study was approved by the Ethical Committee of the University Hospital Leuven and informed consent was obtained from all patients.

Analytic methods

Urea nitrogen, creatinine, and phosphate were measured by standard laboratory techniques. β2m was quantitated by rate nephelometry using an Immage Instrument (Beckman Coulter, Brea, CA, USA). p-Cresol was analyzed by gas chromatography mass spectrometry (GC-MS) technology. Five hundred μL of serum was diluted with 450 μL water. The pH of a 950 μL sample (diluted serum, urine or dialysis fluid) was adjusted to pH 1 with concentrated H2SO4 and the solution was heated to 90°C for 30 minutes. After a cooling down period to ambient temperature, 50 μL 2,6-dimethylphenol solution (20 mg/100 mL) was added as internal standard. One milliliter ethyl acetate was added for the extraction of p-cresol. The solution was well mixed during 30 seconds and centrifuged at 3300 rpm for 20 minutes. Then, 500 μL of the supernatant was dried over anhydrous sodium sulfate and 100 μL of the resultant sample was transferred to the GC-MS (Trace GC-MS, ThermoFinnigan, San José, CA, USA) for automatic splitless injection of 0.5 μL. The analytic column used was a 30 m × 0.32 mm internal diameter, film thickness 1 μm AT5-MS (Alltech, Deerfield, IL, USA). Helium GC grade was used as a carrier gas with a constant flow of 1.3 mL/min. The oven was programmed from 75°C (isotherm for 5 minutes) to 280°C with 15°C per minute. After separation, p-cresol was identified by MS (Electron Impact full scan mode from m/z 59 to m/z 590 at 2 scan/second). Quantitative results were obtained by the internal standard method and calculated as concentrations (mg/L).

Calculations

Peritoneal, renal, and total clearances normalized to 1.73 m² body surface area (BSA) (L/week/1.73 m²) were calculated for all solutes by direct determination from dialysis fluids, urine and mid-day serum solute concentrations. According to the National Kidney Foundation Dialysis Outcomes Quality Initiative (NKF-K-DOQI) guidelines [2], residual glomerular filtration rate (GFR) was estimated by calculating the arithmetic mean of renal urea nitrogen and creatinine clearance and expressed in mL/min/1.73 m². Peritoneal, renal, and total Kt/V were calculated only for urea nitrogen (Kt/V UN). BSA was estimated by the Du Bois and Du Bois method [20]. The distribution volume of urea nitrogen (V) was assessed by the Watson, Watson, and Batt formula for total body water [21]. Protein nitrogen appearance, normalized to body weight (nPNA) was calculated according to Bergström, Heimburger, and Lindholm [22].

Uremic symptom score

A uremic symptom score was developed, using a previously described dyspepsia questionnaire [23–25] with the addition of 12 symptoms. Each patient was asked to grade the intensity (0, absent; 1, mild; 2, relevant; 3, severe and interfering with daily activities) of 20 different symptoms (Table 1). As indicated in the table, the total sum of scores (S tot) was calculated as well as the subtotals for three different symptom classes: gastrointestinal symptoms (Sg), neurological symptoms (S neur), and itching (Sskin).

Statistics

Data are expressed as mean ± SEM. Differences between APD and CAPD were evaluated using unpaired Student t test or Mann-Whitney U test for continuous data and chi-square test of association for categorical data where appropriate. For paired comparisons of different solute clearances, Friedman test with post hoc analysis was used. Pearson correlation coefficients were calculated to assess possible associations between clearances, parameters of renal function, serum solute concentrations, and uremic symptom scores. P values less than 0.05
were considered significant. The SAS version 8.02 (SAS Institute, Cary, NC, USA) software program was used for the statistical analysis.

RESULTS

Patient characteristics
Patient characteristics are summarized in Table 2. Mean age was 52.0 ± 3.0 years. Mean duration of dialysis at the time of the study was 16.2 ± 2.0 months. Mean body weight was 66.5 ± 2.2 kg and mean BSA was 1.75 ± 0.04 m². Mean 24-hour urine output was 1009 ± 120 mL. Mean 24-hour peritoneal drainage was 11658 ± 553 mL. There were no statistically significant differences between APD and CAPD patients in any of the observed parameters. Renal diagnoses were equally distributed between the two groups. There were no differences in the use of diuretics, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers, other antihypertensives, and nonsteroidal anti-inflammatory drugs.

Peritoneal dialysis adequacy
No significant differences were found between APD and CAPD for mid-day serum levels of urea nitrogen, creatinine, phosphate, and β2m. Serum p-cresol was significantly lower in CAPD than in APD patients (P = 0.0049). Peritoneal, renal, total Kt/VUN values, and GFR were not different between the two groups (Table 3).

Table 1. Uremic symptom questionnaire

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Score</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epigastric discomfort worsening with food intake</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Epigastric pain</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Early satiety</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Postprandial fullness</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Bloating</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Nausea</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Belching</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Epigastric burning</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Hiccup</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Sleeping disorders</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Erratic memory</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Concentration disturbances</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Drowsiness</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Cramps</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Restless legs</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Itching</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Indicate the intensity of each of the following symptoms during the previous 3 months: 0 = absent, 1 = mild, 2 = relevant, 3 = severe and interfering with daily activities.

Example of the uremic symptom questionnaire. In the table, total sum of scores and the subtotals according to three symptom classes are indicated. The ranges of possible scores are indicated between parentheses.

DISCUSSION

Our study, comprising 30 ESRD patients treated with peritoneal dialysis, shows that the total and peritoneal clearances of β2m, p-cresol, and phosphate are significantly lower as compared to the clearances of the reference uremic retention solutes urea nitrogen and creatinine (Table 4) (Fig. 1). For β2m this observation is not surprising, since the high molecular weight of the solute (11815 D) hampers its diffusive and convective transport through the pores of the peritoneal membrane [26–28]. The findings are in accordance with earlier studies in children [29, 30] and adults on peritoneal dialysis [31]. The low clearances of p-cresol (108 D) represent a new finding. They are not unexpected, however, given the
Our clearance data demonstrate that the elimination of \( \beta_2m \) and p-cresol depends largely on residual renal function (Table 4) (Fig. 1). The importance of renal elimination is further stressed by the inverse relationship found between serum levels of both molecules and parameters of renal function (24-hour urine volume, renal \( \text{Kt/V}_{\text{UN}} \), and GFR). For p-cresol this relationship lacks statistical significance, which can probably be attributed to the fact that the generation rate of the molecule, in contrast to that of \( \beta_2m \) [34–36], can vary substantially within and between individuals. The amount of ingested protein escaping small intestinal digestion and absorption, the characteristics of the bacterial flora and the colonic transit time were all shown to influence p-cresol generation [37–39]. Variations of these factors might obscure the relationship between serum levels of the solute and its renal elimination.

Peritoneal clearance values of urea nitrogen, creatinine, and phosphate were inversely correlated with their respective renal clearances, which was not the case for \( \beta_2m \) and p-cresol. This observation further underlines the importance of residual renal function for the elimination of the latter molecules, since a loss of renal clearance seems not to be compensated by an increase in peritoneal clearance.

Several recent studies indicate the value of residual renal function as a predictor of patient survival in peritoneal dialysis patients [4–7]. This has been explained by its role in the maintenance of an adequate fluid balance [8–11]. In view of our findings, the conservation of renal elimination mechanisms other than glomerular filtration [13, 14] might contribute substantially to the explanation of residual renal function as an outcome predictor.

The finding of low clearance values of phosphate (96 D) is in accordance with earlier reports illustrating the middle molecule characteristics of the solute [40–42]. Several explanations for this particular behavior have been formulated. First, due to its hydrophilic characteristic, the phosphate molecule is surrounded by an aqueous cover. Moreover, up to 40% of the circulating phosphate has been shown to be a component of sodium, calcium, and magnesium salts [40]. Finally, phosphate is mainly distributed in the intracellular space with a slow intra- and extracellular solute transfer rate [43].

We found significant differences in peritoneal clearances of \( \beta_2m \) and p-cresol between APD and CAPD. This observation corroborates the results of a recent study, which showed that in contrast to small water-soluble molecules, the peritoneal clearance of \( \beta_2m \) depends mainly on the total dwell hours of peritoneal dialysis and not on the number of exchanges of dialysis fluid [31]. Moreover, although not statistically significant, the 24-hour ultrafiltration volume was higher in CAPD than in APD patients, which might have contributed to a greater solute removal by convection in the former. On the other hand, the duration of dialysis at the time of our study was shorter in the CAPD patients (not statistically significant). This may have biased our results, since the
peritoneal membrane restriction coefficient for macromolecules has been demonstrated to increase in relation to the years on peritoneal dialysis treatment [30, 44]. Serum levels of p-cresol were significantly lower in CAPD than in APD patients. This finding most probably reflects the differences in peritoneal clearance of the molecule, since no differences between the two groups were seen in parameters of renal function (24-hour urine output, renal Kt/V \( \text{UN} \), or GFR) or in dietary protein intake (nPNA).

An interesting finding of our data is the positive correlation between serum levels of p-cresol and a uremic symptom score, based on a validated dyspepsia questionnaire [23–25]. To our knowledge, this is the first evidence of a clinical end point related to the protein-bound retention product in peritoneal dialysis patients. One might argue that this relation is self-evident, since p-cresol is just one of the numerous retention solutes in uremia and might play the role of “innocent bystander.” However, p-cresol was the only of the five toxins studied revealing a significant correlation with the symptom score. Moreover, there was a trend to lower symptom scores in the CAPD patients in conjunction with lower serum levels and higher peritoneal clearances of p-cresol as compared to the APD patients. These observations further suggest that protein bound solutes play a role in the pathophysiology of uremic symptoms.

We recognize that the serum levels of p-cresol found by our laboratory are higher than most other values reported in the literature [45, 46]. A possible explanation for this discrepancy lies in the sample preparation, which consists essentially of a deproteinization and an extraction step. In addition to acid deproteinization, which is reported in the literature [45, 46]. A possible explanation for this discrepancy lies in the sample preparation, which consists essentially of a deproteinization and an extraction step. In addition to acid deproteinization, which is applied in most other studies, we used heat denaturation. It is not excluded that the high temperature used in this setting not only deproteinizes the serum but, in addition, provokes hydrolysis of circulating conjugates of p-cresol (p-cresylsulfate and p-cresylglucuronide). Although differences in concentration measurements would probably have no impact on clearance calculations, further research is needed to elucidate this topic.

**CONCLUSION**

Our data show clear evidence for the middle molecule characteristics of the protein-bound solute p-cresol during peritoneal dialysis. As for \( \beta_2 \text{m} \), its total and peritoneal clearances were shown to be lower than those of the small water-soluble solutes urea nitrogen and creatinine. Moreover, the importance of residual renal function in the elimination of \( \beta_2 \text{m} \) and p-cresol was demonstrated. Finally, our study is supportive for the involvement of protein-bound solutes in the pathophysiology of uremic symptoms.

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