The relationship between effluent potassium due to cellular release, free water transport and CA125 in peritoneal dialysis patients

Annemieke M. Coester¹, Machteld M. Zweers¹, Dirk R. de Waart² and Raymond T. Krediet¹

¹Division of Nephrology, Department of Medicine and ²Department of Experimental Hepatology, Academic Medical Centre, University of Amsterdam, The Netherlands

Abstract

Background. Recently, we found evidence of effluent potassium (K⁺) additional to diffusion and convection, suggesting cellular release (CR). Its relationship with free water transport (FWT) in stable peritoneal dialysis (PD) patients suggested an effect of hypertonicity of the dialysis solution leading to cell shrinkage. The aim of the present study was to reproduce these findings in groups according to PD duration and to further investigate the role of mesothelial cells in the observed phenomenon.

Methods. Standard peritoneal permeability analyses done with 3.86% glucose were analysed cross-sectionally in three different groups: short-term (n = 53) 0–2 years PD treatment; medium-term (n = 24) 2–4 years PD and long-term (n = 26) > 4 years PD.

Results. The time courses for FWT and cellular release of K⁺ (CR-K⁺) during the dwell were not significantly different among the groups. Cancer antigen (CA) 125 was highest in the short-term group (P ≤ 0.02) and had a strong positive correlation with mass transfer area coefficient of creatinine (MTAC-creatinine) only in the short-term group (r = 0.62, P ≤ 0.01). CA125 had no relationship with either CR-K⁺ or FWT, except for negative relationships in the short-term group (CR-K⁺, r = −0.41, P ≤ 0.05; FWT, r = −0.54, P ≤ 0.05).

Conclusion. We conclude that the correlation of CA125 and MTAC-creatinine is dependent on PD duration and underlines the in vitro observation that mesothelial cells produce vasoactive substances that may increase the peritoneal surface area. However, CA125 is not directly related to CR-K⁺ or FWT. Therefore, the relationship between FWT and CR-K⁺ is likely to reflect hypertonic cell shrinkage, regardless of the duration of PD, and confirms our earlier findings.

Introduction

Recently, we found evidence of effluent potassium (K⁺) additional to diffusion and convection in peritoneal dialysis (PD) patients [1]. Its relationship to free water transport (FWT) in patients without ultrafiltration (UF) failure suggested an effect of hypertonicity of the dialysis solution: the glucose-induced crystalloid osmotic pressure draws water out of the cell and K⁺ follows [1]. Endothelial cells were considered the most likely source for this phenomenon due to the location of the aquaporin-1 water channel, especially in capillary and venular endothelium [2]. However, mesothelial cells also have aquaporins located in their cell membrane [2]. The influence of other cells on additional release of K⁺ and its relationship with FWT during PD are not known.

Cancer antigen (CA) 125 is constitutively produced by peritoneal mesothelial cells in vitro [3–5]. It can reflect mesothelial cell mass [6] and correlates with the number of mesothelial cells in the peritoneal effluent of stable PD patients [3,4,7]. The aim of the present study is to analyse whether the findings of our previous study can be reproduced in three different groups according to the duration of PD and to further investigate the role of mesothelial cells in the observed phenomenon.

Subjects and methods

Standard peritoneal permeability analyses (SPAs) of 103 clinically stable patients were investigated cross-sectionally and divided into three groups: short-term: <2 years PD (n = 53); medium-term: 2–4 years PD (n = 24) and long-term: >4 years PD (n = 26). Patients were not selected for the presence or absence of UF failure. All patients were free of peritonitis for at least 4 weeks prior to the test and used commercially available glucose based dialysis solutions (Baxter Healthcare Ltd, Ireland).

Subjects

In the short-term group, the median age was 56 years (range 19–77) and median net UF 548 mL/4 h (−265 to 1169).
In the medium-term group, the median age was 46 years (18–74) and median net UF 581 mL/4 h (134–999). In the long-term group, the median age was 44 years (20–73) and median net UF 581 mL/4 h (–659 to 1035). Median PD duration is shown in Table 1.

**Procedure**

All SPAs were done during a 4-h dwell with 3.86%-glucose-based dialysis solutions (Dianeal®). Baxter Healthcare Ltd), as previously described [7]. Briefly after a rinsing procedure, a fresh 3.86%-glucose-based dialysis solution was instilled for a test dwell. Effluent samples were taken at 0, 10, 20, 30, 60, 120, 180 and 240 min. Blood samples were taken at the beginning and end of the SPA. Dextran 70 (Hyskon®, Medisan Pharmaceuticals AB, Uppsala, Sweden) 1 g/L was added to calculate peritoneal fluid kinetics. To prevent possible anaphylactic reactions to dextran 70, dextran 1 (Promiten®, NPBI, Emmercompascuum, The Netherlands) was given intravenously before instillation [9].

**Assays**

Effluent CA125 was determined in the 4-h effluent by a micro-particle enzyme immunoassay (MEIA) (Abbott Laboratories, North Chicago, IL, USA) using a commercially available monoclonal antibody OC125 (Fujirebio Diagnostics, Inc., Malvern, PA, USA) on an IMx auto analyser. This assay has a low detection limit of 0.4 U/mL and is similar to the one previously used [3,4,7,10,11]. Ion selective electrodes were used to measure Na⁺ and K⁺. Glucose was assessed by the glucose oxidase–peroxidase method with an auto analyser (Hitachi 747, Boehringer Mannheim, Germany). Total protein in plasma was determined by the biuret method (Roche, Almere, The Netherlands), also with the automated analyser. Beta2-Microglobulin (β2-microglobulin) was determined with an IMx system, also applying a MEIA. Ion selective electrodes were used to measure Na⁺ and K⁺. Total dextran concentration in effluent was determined by high-performance liquid chromatography [12].

**Calculations of solute and fluid transport**

Solute and fluid transport parameters were calculated as mass transfer area coefficients (MTAC) [13]. Solute concentrations in serum were corrected for plasma water [14]. Glucose absorption was calculated as the difference between the amount of glucose instilled and recovered, relative to the amount instilled.

Transcapillary UF (TCUF) comprises water transport through small interendothelial pores (SPT) and ultra-small transendothelial pores, so-called FWT. The amount across the large pores is considered negligible. Changes in intraperitoneal volume (ΔIPV) result from TCUF and fluid reabsorption, which includes lymphatic absorption, disappearance into the interstitial tissues (together effective lymphatic absorption, ELA) and back-filtration into the capillaries. In our model, ΔIPV or NUF is TCUF minus ELA. NUF was calculated as the difference between the IPV at the end of the dwell and the initial IPV. TCUF was calculated from the dilution of the intraperitoneal administered volume marker by subtracting the initial IPV from the theoretical IPV (when both fluid absorption and sampling were not present).

FWT was calculated as described previously [1] by subtracting TCUF coupled with Na⁺ transport [fluid transport through small pores (SPT)] from TCUF. FWT and SPT are expressed as absolute values. A diffusion correction for sodium (Na⁺) sieving was performed using the MTAC of urate [2].

**Calculations on cellular release (CR) of K⁺**

For exact calculation we refer to our previous study [1]. In brief, it is based on the least-squares regression analysis of the D/P ratios of urea, creatinine, urate and β2-microglobulin, and their free diffusion coefficients when plotted on a double logarithmic scale. This results in a diffusion/transport line from which the expected value due to diffusion can be calculated. Effluent K⁺ additional to diffusion is defined as the difference between the measured and the expected D/P ratio. The regression coefficients exceeded 0.93 (P < 0.05) in 93 of the patients. The measured D/P K⁺ exceeded the expected D/P K⁺ in all these patients. D/P ratios at all time points during the dwell were used to calculate cellular release of K⁺ (CR-K⁺).

**Statistical analysis**

Data are presented as means ± SD, unless stated otherwise. ANOVA with Bonferroni correction was used to compare the normally distributed continuous parameters.
The relationship between effluent potassium, free water transport and CA125

The Kruskal–Wallis and Mann–Whitney U-tests were applied for the asymmetrically distributed data. Pearson and Spearman correlation analyses were used to analyse possible relationships. Linear mixed model analysis was used to describe the time course of CR-K⁺, FWT and SPT during the dwell.

**Results**

Peritoneal solute and fluid transport characteristics of the groups are summarized in Table 1. CA125 values are shown in Figure 1. Figure 2 shows the fluid profile during the dwell for FWT (upper panel) and the time course for CR-K⁺ during the dwell (lower panel). The time course for SPT showed a gradual increase during the dwell, but no difference among the groups \((P = 0.5)\). Highest values were present in the short-term group. Like FWT, lowest values were present in the long-term group.

**Correlations**

Table 2 shows the Pearson correlation coefficients of the investigated parameters. Mostly all correlations between CR-K⁺ and FWT at different time points were significant. No significant relationships were present between CR-K⁺ and SPT in any of the groups, except for the short-term group at 240 min. Significant negative relationships were present between CA125 and both CR-K⁺ \((r = -0.41, P \leq 0.05)\) and FWT \((r = -0.54, P \leq 0.05)\) in the short-term group at 240 min. There were no relationships with CA125 in the medium-term group \((CR-K^+; r = -0.16, P = 0.5; FWT: r = -0.34, P = 0.1)\) and the long-term group \((CR-K^+; r = 0.13, P = 0.5; FWT: r = 0.21, P = 0.3)\). CA125 had a strong positive correlation with MTAC-creatinine in the short-term group \((r = 0.62, P \leq 0.01)\), but not in the medium-term \((r = -0.03, P = 0.9)\) and long-term groups \((r = 0.19, P = 0.3)\). MTAC-creatinine had strong negative relationships with FWT in all the groups \((r = -0.57 to -0.60, all P \leq 0.01)\), but weaker associations with CR-K⁺ \((short-term, r = -0.29, P = 0.04; medium-term, r = -0.18, P = 0.4; long-term, r = -0.45, P = 0.03)\).

A sub-analysis was performed in which patients with UF failure [17] were withdrawn from the analysis: 13 patients in the short-term, 4 in the medium-term and 9 in the long-term group had UF failure. Similar relationships prevailed (data not shown). Due to the limited number of UF failure patients per group, it was not relevant to analyse these groups separately.

**Table 2.** Pearson correlations of cellular release of K⁺ in relation to free water transport at either 60 or 240 min of the dwell

|                    | Short-term group   | Medium-term group | Long-term group |
|--------------------|--------------------|-------------------|-----------------|
| FWT at 60 min (mL) | 0.41**             | 0.30              | 0.39*           |
| FWT at 240 min (mL)| 0.54**             | 0.60**            | 0.57**          |
| SPT at 60 min (mL) | 0.10               | 0.17              | 0.23            |
| SPT at 240 min (mL)| 0.22*              | 0.11              | 0.16            |

* \(P \leq 0.05\), ** \(P \leq 0.01\). For abbreviations see Table 1.
Discussion

The findings of the present study indicate that the phenomenon of hypertonic cell shrinkage is present in stable PD patients and is not dependent on the duration of PD treatment. Its relationship with free water transport in patients without UF failure suggested an effect of hypertonicity of the dialysis solution: the glucose-induced crystalloid osmotic pressure draws water out of the cell and K⁺ follows [1]. This confirms the results of our earlier study [1] where positive correlations between CR-K⁺ and FWT were found in patients who had PD treatment for <1 year and >4 years without UF failure. In the present study also strong positive correlations were present, most obvious in the short-term and long-term groups. This may have been due to the presumed absence of marked anatomical changes in the short-term group [18], and functional stability [19,20] and/or selective dropout in the long-term group. In the medium-term patients, a positive trend with a wide scatter existed for the relationship at 60 min, even after exclusion of the patients with UF failure. Consequently, the case mix of the patient population studied in the medium-term group, particularly with regard to the development of peritoneal membrane alterations, is likely to determine whether CR-K⁺ has a correlation with FWT.

Previously, a study by Parikova et al. [21] found a U-shaped tendency for the MTAC-creatinine. The initial high values suggest the influence of vasoactive substances [22], whereas the subsequent rise suggests the development of peritoneal membrane alterations [19,20]. Ha et al. [23] and others [24] have shown evidence of mesothelial production of vasoactive substances, like vascular endothelial growth factor (VEGF). A study in incident PD patients suggested a role of VEGF in the regulation of the peritoneal vascular surface area where VEGF influenced the relationship between MTAC-creatinine and CA125 [24]. In the present study, the MTAC-creatinine had the highest values in the short-term and long-term groups and lowest values in the medium-term group, although such a strong U shape was not found in the present group of patients. These results and those of Rodrigues et al. [25] support the in vitro finding that the mesothelium produces vasoactive substances that, in the initial phase of PD, can increase the effective peritoneal vascular surface area, i.e. the number of perfused peritoneal capillaries and/or their diameter. Long-term PD is likely to be associated with a decrease in mesothelial cell mass [10] and an increase in the anatomic vascular surface area due to angiogenesis [26]. This explains the lack of correlations between effluent CA125 and the parameters of peritoneal solute transport in the long-term patients. Consequently, the case mix of the population studied, particularly with regard to PD duration, will determine whether a relationship between CA125 and transport parameters is found. This explains the controversial results reported in other cross-sectional studies [11,24–26].

The objective of this study was also to further investigate the role of mesothelial cells in the observed phenomenon of hypertonic cell shrinkage. The inverse relationships between CA125 and both FWT and CR-K⁺ in the short-term group, and the absence in the medium-term and the long-term group suggest that mesothelial cells themselves do not contribute significantly to the phenomenon of hypertonic cell shrinkage. However, a study by Breborowicz et al. [28] reported that mesothelial cells can undergo a rapid volume change when exposed to hypertonic dialysis solutions. It can therefore be questioned as to what extent in vitro results are translational to the clinical situation, also because mesothelial cells are likely to possess adaptation processes to long-term exposure to a hypertonic environment [28]. Our finding makes the vascular endothelial cell a more important source and is in agreement with the location of the aquaporin-1 channels especially in capillary and venular endothelium [2]. Our results are also in line with the findings that the mesothelium forms no hindrance to peritoneal transport [29].

We conclude that the correlation of CA125 and MTAC-creatinine is dependent on PD duration and underlines the in vitro observation that mesothelial cells produce vasoactive substances that may increase the peritoneal surface area. However, CA125 is not directly related to CR-K⁺ and FWT. Therefore, the relation between FWT and CR-K⁺ is likely to reflect hypertonic cell shrinkage regardless of the duration of PD, and confirms our earlier findings.

Acknowledgements. This study was supported by a grant of the Dutch Kidney Foundation—C06.2186. We highly appreciate the excellent data collection by Mr Harald Hutten.

Conflict of interest statement. None declared.

References

1. Coester AM, Struijk DG, Smit W et al. The cellular contribution to effluent potassium and its relation with free water transport during peritoneal dialysis. Nephrol Dial Transplant 2007; 22: 3593–3600
2. Goffin E, Combet S, Jamar F et al. Expression of aquaporin-1 in a long-term peritoneal dialysis patient with impaired transcellular water transport. Am J Kidney Dis 1999; 33: 383–388
3. Koomen GC, Betjes MG, Zemel D et al. Dialysate cancer antigen (CA) 125 is a reflection of peritoneal cell mass in CAPD patients. Perit Dial Int 1994; 14: 132–136
4. Visser CE, Brouwer-Steenbergen JJ, Betjes MG et al. Cancer antigen 125 is a mesothelial bulk marker for mesothelial mass in stable peritoneal dialysis. Nephrol Dial Transplant 1995; 10: 64–69
5. Zeilemaker AM, Verbrugh HA, Hoynck van Papendrecht AA et al. CA 125 secretion by peritoneal mesothelial cells. J Clin Pathol 1994; 47: 263–265
6. Krediet RT. Dialysate cancer antigen 125 concentration as marker of peritoneal membrane status in patients treated with chronic peritoneal dialysis. Perit Dial Int 2001; 21: 560–567
7. Sanussi AA, Zweers MM, Weening JJ et al. Expression of cancer antigen 125 by peritoneal mesothelial cells is not influenced by duration of peritoneal dialysis. Perit Dial Int 2001; 21: 495–500
8. Pannekeet MM, Imholz AL, Struijk DG et al. The Standard Peritoneal Permeability Analysis: a tool for the assessment of peritoneal permeability characteristics in CAPD patients. Kidney Int 1995; 48: 866–875
9. Renock H, Ljungstrom HG, Hedin H et al. Prevention of dextran induced anaphylactic reaction by hapten inhibition. Acta Chir Scand 1983; 149: 355–360
10. Ho-Dac-Pannekeet MM, Hiralall JK, Struijk DG et al. Longitudinal follow-up of CA125 in peritoneal effluent. Kidney Int 1997; 51: 888–893
The relationship between effluent potassium, free water transport and CA125

11. Pannekeet MM, Koomen GC, Struijk DG et al. Dialysate CA125 in stable CAPD patients. *Clin Nephrol* 1995; 44: 248–254

12. Koomen GC, Krediet RT, Leegwater AC et al. A fast reliable method for the measurement of intraperitoneal dextran 70, used to calculate lymphatic absorption. *Adv Perit Dial* 1991; 7: 10–14

13. Waniwski J, Werynski A, Heimburger A et al. Simple models for description of small solute transport in peritoneal dialysis. *Blood Purif* 1991; 9: 129–141

14. Waniwski J, Heimburger A, Werynski A et al. Aqueous solute concentrations and evaluation of mass transfer area coefficients in peritoneal dialysis. *Nephrol Dial Transplant* 1992; 7: 50–56

15. Smit W, Struijk DG, Ho-Dac-Pannekeet MM et al. Quantification of free water transport in peritoneal dialysis. *Kidney Int* 2004; 66: 849–854

16. Zweers MM, Splint LJ, Struijk DG et al. Correction of sodium sieving for diffusion from the circulation. *Adv Perit Dial* 1999; 15: 65–72

17. Mujais S, Nolph K, Gokal R et al. International Society for Peritoneal Dialysis Ad Hoc Committee on Ultrafiltration Management in Peritoneal Dialysis. Evaluation and management of ultrafiltration problems in peritoneal dialysis. *Perit Dial Int* 2000; 20(Suppl 4): S5–S21

18. Zweers MM, Splint LJ, Krediet RT et al. Ultrastructure of basement membranes of peritoneal capillaries in a chronic peritoneal infusion model in the rat. *Nephrol Dial Transplant* 2001; 13: 651–654

19. Davies SJ, Philips L, Naish PF et al. Peritoneal glucose exposure and changes in membrane solute transport with time on peritoneal dialysis. *J Am Soc Nephrol* 2001; 12: 1046–1051

20. Selgas R, Fernandez-Reyes MJ, Bajo MA et al. Functional longevity of the human peritoneum: how long is continuous peritoneal dialysis possible? *Am J Kidney Dis* 1994; 23: 64–73

21. Parikova A, Smit W, Struijk DG et al. The contribution of free water transport and small pore transport to the total fluid removal in peritoneal dialysis. *Kidney Int* 2005; 68: 1849–1856

22. Pecoits-Filho R, Araujo MR, Lindholm B et al. Plasma and dialysate IL-6 and VEGF concentrations are associated with high peritoneal solute transport rate. *Nephrol Dial Transplant* 2002; 17: 1480–1486

23. Ha H, Cha MK, Choi HN et al. Effects of peritoneal dialysis solutions on the secretion of growth factors and extracellular matrix proteins by human peritoneal mesothelial cells. *Perit Dial Int* 2002; 22: 171–177

24. van Esch S, Zweers MM, Jansen MA et al. Determinants of peritoneal solute transport rates in newly started nondiabetic peritoneal dialysis patients. *Perit Dial Int* 2004; 24: 554–561

25. Rodrigues A, Martins M, Santos MJ et al. Evaluation of effluent markers cancer antigen 125, vascular endothelial growth factor; and interleukin-6: relationship with peritoneal transport. *Adv Perit Dial* 2004; 20: 8–12

26. Mateijsen MA, Van Der Wal AC, Hendriks PM et al. Vascular and interstitial changes in the peritoneum of CAPD patients with peritoneal sclerosis. *Perit Dial Int* 1997; 19: 517–525

27. Jimenez C, Diaz C, Selgas R et al. Peritoneal kinetics of cancer antigen 125 in peritoneal dialysis patients: the relationship with peritoneal outcome. *Adv Perit Dial* 1999; 15: 36–39

28. Brøbrovics A, Polubinska A, Oreopoulou DG. Changes in volume of peritoneal mesothelial cells exposed to osmotic stress. *Perit Dial Int* 1999; 19: 119–123

29. Flessner M, Henegar J, Bigler S et al. Is the peritoneum a significant transport barrier in peritoneal dialysis? *Perit Dial Int* 2003; 23: 542–549

*Received for publication: 19.2.08
Accepted in revised form: 19.6.08*