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

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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 ($P \leq 0.02$) and had a strong positive correlation with mass transfer area coefficient of creatinine (MTAC-creatinine) only in the short-term group ($r = 0.62$, $P \leq 0.01$). CA125 had no relationship with either CR-K^+ or FWT, except for negative relationships in the short-term group (CR-K^+, $r = -0.41$, $P \leq 0.05$; FWT, $r = -0.54$, $P \leq 0.05$).

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).

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Table 1. A comparison of peritoneal solute and fluid characteristics between the groups

	Short-term, 5 (1–22) months PD	Medium-term, 29 (24–47) months PD	Long-term, 61 (48–195) months PD	Overall *P*-value
TCUF at 60 min (mL)	494 ± 159	476 ± 99	$397 \pm 167^*$	0.03
TCUF at 240 min (mL)	961 ± 248	982 ± 154	862 ± 301	0.2
SPT at 60 min (%)	70 ± 10	68 ± 10	69 ± 15	0.7
FWT at 60 min (%)	30 ± 10	32 ± 10	31 ± 16	0.6
MTAC-creat. (mL/min)	9.9 ± 3.0	9.8 ± 2.9	$11.7 \pm 4.5^*$	0.05

PD, peritoneal dialysis; TCUF, transcapillary ultrafiltration; SPT, small pore transport; FWT, free water transport; MTAC-creat, mass transfer area coefficient of creatinine.
Months on PD is expressed as medians and ranges. For the other parameters means $\pm$ SD are given.
$^*P \le 0.05$ short-term versus long-term group.

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 535 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 (SMA-II; Technicon, Terrytown, NJ, USA). Urea, creatinine and urate were measured by enzymatic methods with an automated 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 (β_2-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 con-

centrations 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 β_2-microglobulin, and their free diffusion coefficients when plotted on a double logarithmic scale. This results in a diffusion/transportline 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 $\pm$ SD, unless stated otherwise. ANOVA with Bonferroni correction was used to compare the normally distributed continuous parameters.
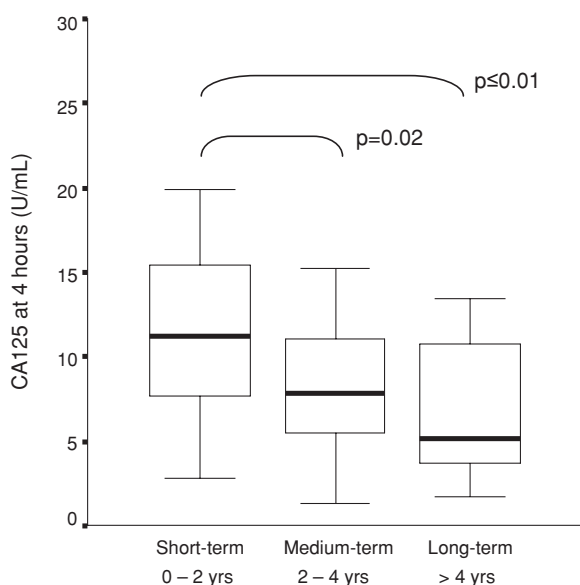
Fig. 1. This figure shows a Box and Whisker plot for cancer antigen 125 at 4 h (U/mL). Data were distributed asymmetrically. Significant higher values were present in the short-term group compared to the other groups.

The Kruskall–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
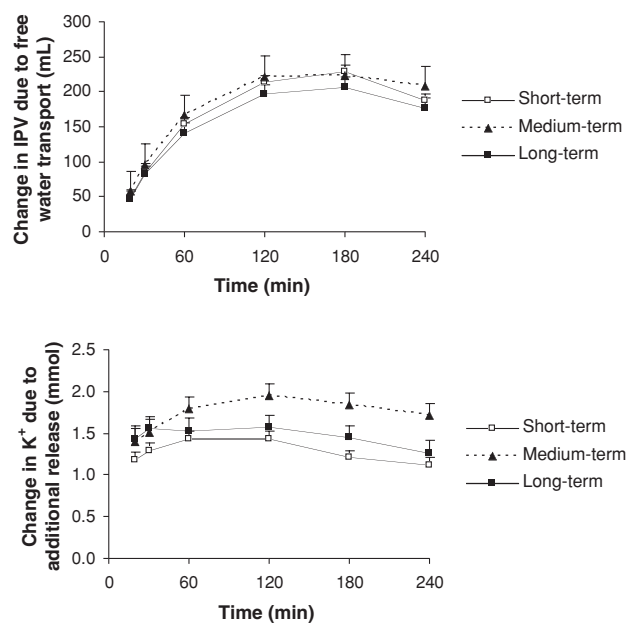
Fig. 2. The upper panel shows the free water transport (FWT) profiles of the three groups during the dwell. During the initial phase of the dwell, FWT increased and levelled off after 120 min of the dwell. The time course during the dwell was not significantly different among the groups ($P = 0.5$). Highest values for FWT during the dwell were present in the medium-term group. Lowest values were present in the long-term group. The lower panel shows the time course for the amount of potassium additional to diffusion. For all groups, an initial increase was followed by a subsequent decrease with highest values ~60–120 min of the dwell. No significant differences were present among the groups ($P = 0.07$), but highest values were present in the medium-term group and lowest values in the short-term group.

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.

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.

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.

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