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High-dose steroid treatment increases free water transport in peritoneal dialysis patients

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Abstract

The water channel aquaporin-1 (AQP1) is the molecular counterpart of the ultrasmall pore that mediates free water transport during peritoneal dialysis (PD). Proof-of-principle studies performed in rats have shown that treatment with corticosteroids upregulates the expression of AQP1 in the peritoneal capillaries, causing a significant increase in free water transport. Whether such a beneficial effect could be observed in end-stage renal disease patients treated by PD remains unknown. Peritoneal transport parameters were evaluated in three patients on PD, shortly before and after living-donor renal transplantation and treatment with high-dose methylprednisolone (1.0–1.2 g/m^2). As compared with pre-transplantation values, the post-transplantation test revealed an ~2-fold increase in the sodium sieving and ultrasmall pore ultrafiltration volume, suggesting an effect on AQP1 water channels. In contrast, there was no change in the parameters of small solute transport. The direct involvement of AQP1 in these changes is suggested by the expression of glucocorticoid receptors in the human peritoneum and the presence of conserved glucocorticoid response elements in the promoter of the human *AQP1* gene.

Keywords: aquaporin-1; endothelium; peritoneal capillaries; sodium sieving

Introduction

The capacity for ultrafiltration (UF) across the peritoneal membrane is a major predictor of outcome and mortality for end-stage renal disease (ESRD) patients treated with peritoneal dialysis (PD) [1, 2]. The major barrier for the transport of water and solutes across the peritoneal membrane is the

endothelium lining peritoneal capillaries. Modelling of peritoneal transport has predicted that endothelial cells contain ultrasmall pores (radius < 3 Å) that facilitate the osmotic transport of water during a hypertonic dwell [3]. The ultrasmall pores account for half of the UF and their patency explains the 'sodium sieving', i.e. the marked fall of the dialysate to plasma ratio of sodium during the first hour of PD [3]. Several lines of evidence have demonstrated that the water channel aquaporin-1 (AQP1), which is abundantly expressed in the endothelial cells lining peritoneal capillaries and veinules [4], is the molecular counterpart of the ultrasmall pores ([5], for review). Studies in *Aqp1*-null mice demonstrated that AQP1 facilitates water transport and mediates approximately half of the UF during crystalloid osmosis [6, 7]. Accordingly, increasing the expression of AQP1 in the peritoneum could improve water transport in PD patients. Based on the presence of glucocorticoid response elements (GREs) in the promoter of the murine AQP1 gene [8], Stoenoiu *et al.* [9] demonstrated that treatment with corticosteroids increases the expression of AQP1 in peritoneal capillaries, mirrored by increased water transport and UF, without affecting the transport of small solutes. Thus far, it is unknown whether a similar effect could be evidenced in patients undergoing PD. We report the serial determination of water and solute transport parameters across the peritoneal membrane in three PD patients that received high-dose glucocorticoids as standard care after a living-donor renal transplantation.

Materials and methods

The clinical features of the three PD patients are detailed in Table 1. 'Patient 1' is an 11-year-old girl who presented haemolytic uraemic syndrome at 18 months of age, progressing to ESRD at the age of 10 years. She was initially treated by continuous ambulatory peritoneal dialysis (CAPD) for 6 months, followed by automated peritoneal dialysis for 4 months. 'Patient 2' is a 54-year-old male with autosomal dominant polycystic kidney disease progressing to ESRD at the age of 52 years. He was treated by CAPD for 9 months before transplantation. 'Patient 3' is a 6-year-old boy who reached ESRD at the age of 5 years for congenital vesico-ureteral reflux. He was treated by CAPD for 5 months. The course of PD was uncomplicated in the three patients. They underwent a living-donor renal transplantation after a mean of 8 months (range: 5–10 months) of PD. The renal transplantation was uneventful, with immediate graft function in the three cases. The details of cumulative methylprednisolone dose and immunosuppressive treatment are given in Table 1. The peritoneal function was assessed in the three patients using the mini peritoneal equilibration testing (mini-PET) [10] performed before (range: 1 day to 3 months) and after (range: 7–39 days) renal transplantation. The sodium concentration was measured by indirect ion-selective analysis (Modular Analytics Evo 900, calibrated by ISE compensator; Roche Diagnostics, Buenos Aires, Argentina). The study was approved by the Ethical Review Board and informed consent was obtained.

The expression of the glucocorticoid receptor (GR) messenger RNA was assessed in human peritoneal biopsies using reverse transcription–polymerase chain reaction (RT–PCR) [9]. Total RNA was extracted from two human peritoneal biopsies (obtained at time of catheter removal in PD patients, ref. [4]) using Aurum™ Total RNA Tissue Kit (Bio-Rad, Hercules, CA), following the manufacturer's protocol, followed by DNAse I treatment to eliminate genomic DNA contamination. Total RNA was extracted from human erythroleukemia cells (positive control for GR expression) using RNAqueous®-Micro kit (Ambion). One microgram of RNA was used to perform the reverse transcriptase reaction with iScript™ cDNA Synthesis Kit (Bio-Rad). RT–PCR detection was performed with FastStart Taq DNA Polymerase (Roche) for 32 cycles, and polymerase chain reaction products visualized on a 1.5% agarose gel with GelRed dye (Sigma). The primers were designed using Beacon Design 2.0 (Premier Biosoft International, Palo Alto, CA): glyceraldehyde 3-phosphate dehydrogenase (forward 5′-3′: GGG GCT CTC CAG AAC ATC AT and reverse 5′-3′: TCT AGA CGG CAG GTC AGG T) and GR (forward 5′-3′: ACA CTG CCC CAA GTG AAA AC and reverse 5′-3′: CCA TGA ACA GAA ATG GCA GA).

Immunostaining was performed on paraffin-embedded sections of human peritoneal biopsies by using a sequential staining protocol [4]. Sections were

Table 1. Clinical and peritoneal equilibration test (mini-PET) parameters in the three patients[a]

	Patient 1		Patient 2		Patient 3	
Gender, age (years)	Female, 11		Male, 54		Male, 6	
Cause of ESRD	Haemolytic uraemic syndrome		ADPKD		Congenital reflux	
Age at ESRD (years)	10		52		5	
Duration of PD (months), modality	10, CAPD (6) then APD (4)		9, CAPD		5, CAPD	
Residual diuresis on PD (mL/day)	500		1000		500	
Time of pre-transplant mini-PET (days)	1 day		90 days		2 days	
Methylprednisolone dose (g/m^2)[b]	1.0		1.0		1.2	
Other (immunosuppressive) drugs introduced per transplantation	Daclizumab (1 mg/kg); tacrolimus (0.15 mg/kg/day); MMF (1 g/day)		Tacrolimus (0.15 mg/kg/day); MMF (2 g/day)		Daclizumab (1 mg/kg); tacrolimus (0.15 mg/kg/day); MMF (1 g/day)	
Time to post-transplant mini-PET (days)	7		20		39	
Serum creatinine at post-transplant (mg/dL)	0.44		1.2		0.43	
mini PET	Pre-TP	Post-TP	Pre-TP	Post-TP	Pre-TP	Post-TP
Sodium sieving (%)[c]	8.3	11.4	5.4	13.1	4.4	12.7
Sodium dip (mmol/L)[d]	11	15	7	17	6	17
UF USP (mL)	126	150	170	332	30	90
UF 1 h (mL)	240	180	620	380	60	180
D/P creatinine	0.40	0.47	0.54	0.46	0.66	0.66
Volume infused (L/m^2)	1.1	1.1	1.2	1.2	0.9	0.9

[a]ESRD, end-stage renal disease; APD, automated peritoneal dialysis; MMF, mycophenolate mofetil; PET, peritoneal equilibration test. D/P, dialysate-over-plasma ratio; Post-TP, post-transplantation; Pre-TP, pre-transplantation; USP, ultrasmall pores.
[b]Cumulative dose until post-transplantation mini-PET.
[c]Sodium sieving: $(D_0/P_0 - D_{60}/P_{60})/D_0/P_0 \times 100$ (in %) (ref. [7]).
[d]Sodium dip: absolute dip of dialysate sodium concentration at 60 min of the PET (mmol/L) [(ref. 11)].

incubated with 3% serum for 20 min before adding anti-von Willebrand factor (DAKO, Glostrup, Denmark) diluted in phosphate-buffered saline containing 2% bovine serum albumin for 1 h. After washing, sections were incubated with Alexa633-labelled secondary anti-IgG antibodies (Invitrogen, Carlsbad, Belgium) for 45 min. The secondary staining was performed by adding anti-glucocorticoid receptor (Abcam, Cambridge, UK) and labeled with Alexa488-labeled secondary anti-IgG antibodies before mounting in Prolong Gold Anti-fade reagent (Invitrogen). Sections were viewed under a Zeiss LSM510Meta Confocal microscope (Carl Zeiss, Zaventem, Belgium), using ×20 Plan-Apochromat or ×63/1.4 Plan-Apochromat oil-immersion objectives (Zeiss).

The 3-kb promoter sequence of the human *AQP1* gene was analysed for putative GRE using Transcription Element Search Software (http://www.cbil.upenn.edu/cgi-bin/tess/tess).

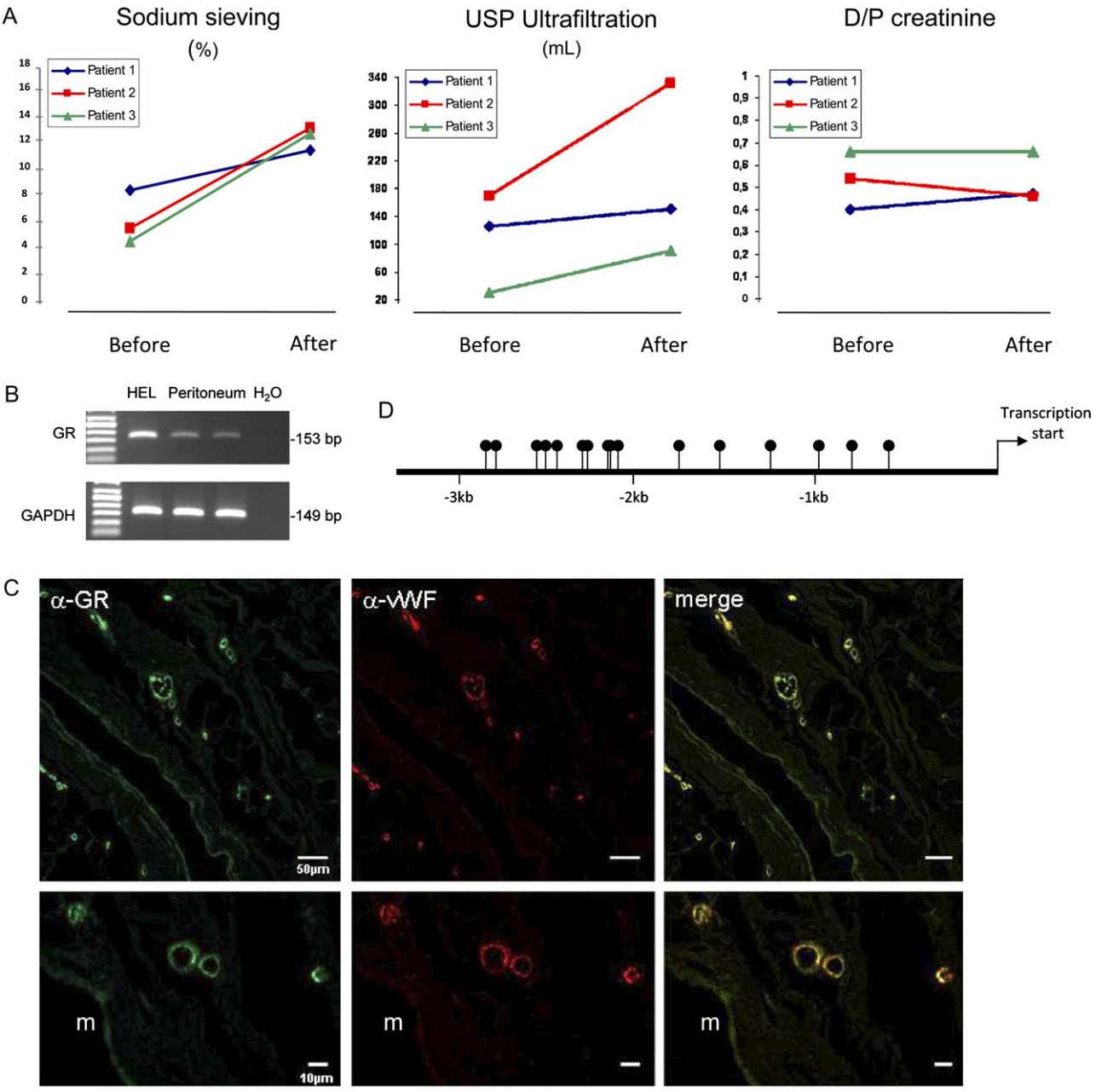

Fig. 1. Transport parameters before and after renal transplantation and expression of the GR in the human peritoneum. (**A**) Comparison of the transport parameters before and after renal transplantation. A mini-PET was performed before and after transplantation in the three patients, who received methylprednisolone at time of transplantation. As compared with baseline values, the three patients show an ~2-fold increase in the sodium sieving and in the ultrasmall pore specific (USP) UF (mL) post-transplantation, whereas the transport of small solutes (D/P creatinine) remains unchanged. Individual values are given in Table 1. (**B**) RT–PCR demonstrating the expression of the GR (153 bp) in two peritoneal biopsies from patients on PD and in the control human erythroleukaemia (HEL) cells. (**C**) Immunolocalization of the GR in human peritoneum. Double immunostaining with anti-glucocorticoid receptor (α-GR) and anti-von Willebrand factor (α-vWF) antibodies show that the GR is expressed in endothelial cells lining the capillaries and small vessels in the peritoneal membrane. Low magnification: bar: 50 µm; detail: 10 µm. m, mesothelium. (**D**) Organization of human *AQP1* gene promoter. The 3-kb proximal promoter of *AQP1* is schematically represented, including the transcription start site and the 16 predicted GREs (vertical symbols).

Results and discussion

The evolution of the peritoneal transport parameters as assessed by the mini-PET is shown on Table 1. As compared with pre-transplantation, the post-transplantation peritoneal equilibration test evidenced an ~2-fold increase in the sodium sieving (and its surrogate marker the absolute dip of dialysate sodium concentration) [11], paralleled by a similar increase in the ultrasmall pore-specific UF. In contrast, the small solute transport rate, as assessed by the D/P creatinine and small pore-specific UF, was unchanged (Figure 1A, Table 1). By analogy with results obtained in the rat [9], the potential role of AQP1 was substantiated by expression studies demonstrating the expression of the GR in the capillary endothelium of the human peritoneum and the 'in silico' identification of conserved GREs in the promoter of the *AQP1* gene (Figure 1B–D).

These data are the first to document a major increase in the parameters of free water transport in PD patients following high-dose glucocorticoid administration in the context of renal transplantation. To the best of our knowledge, there are no previous reports on the effect of renal transplantation *per se* on PD transport parameters. Several points argue in favour of a specific effect of the glucocorticoids on AQP1. Firstly, the time course of the effect was observed to be similar in the three patients who all had a short course of uncomplicated PD followed by a living-donor renal transplantation with immediate renal function. Secondly, the fact that the changes are restricted to the very parameters—sodium sieving, sodium dip and ultrasmall pore-specific UF—that reflect the AQP1-mediated water transport, whereas there is no effect on the D/P creatinine parameter assessing small solute transport. The lack of effect of small solutes is important when considering that exposure to mycophenolate mofetil can, at least *in vitro*, inhibit angiogenesis [12, 13] and thus decrease the exchange area. Thirdly, the fact that no other external factor other than high-dose glucocorticoids has been reported to specifically affect free water transport and expression of AQP1. Fourthly, we provide evidence for multiple GREs in the promoter of the *AQP1* gene and for a strong expression of the GR in the endothelium lining peritoneal capillaries, i.e. in the very cells where AQP1 mediates osmotic water transport during PD. We fully acknowledge the limitations of this preliminary report, including the small number of patients, with only one adult, which is not very representative of the PD population, and the lack of low-dose steroid controls assessing the effect of transplantation (e.g. removing uremia, inducing acute changes in body parameters and putting the peritoneal membrane at rest). These limitations are balanced by the specificity of the changes in free water transport and the lack of effect on other transport parameters. We should also keep in mind the possibility of a role for the interstitium in the water-only fluid pathway [14], although the time course for a dramatic reorganization of the interstitium (except for a reduction in the degree of edema) seems to be too short for such an explanation of the observed changes.

Conclusion

In conclusion, these data provide the first line of evidence supporting the effect of corticosteroids on specific parameters of AQP1-mediated water transport in PD patients. Although these results have to be confirmed in a larger patient cohort, the specificity of the changes in free water transport suggests that pharmacologic induction of AQP1 may help to increase water transport in patients treated by PD.

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Conflict of interest statement. None declared.

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