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Combinatorial Peptide Ligand Library and two dimensional electrophoresis: New frontiers in the study of peritoneal dialysis effluent in pediatric patients

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ABSTRACT

Peritoneal dialysis effluent (PDE) is a fluid resulting from the close contact of peritoneal dialysis (PD) solutions with the peritoneal membrane (PM) and represents a readily available material for the search of biomarkers of PM function or damage.

Our laboratory has developed a method for the in-depth proteomic characterization of PDE, which involves Combinatorial Peptides Ligand Library (CPLL) to reduce the dynamic range of protein concentration in PDE, followed by two-dimensional electrophoresis (2-DE). In this study we applied this method to the analysis of PDE proteome obtained from 19 pediatric patients on automated peritoneal dialysis (APD) with glucose-based PD solutions.

The combined use of this proteomic approach highlighted a mean of 700 new proteins. Differences in PDE proteome profile were observed in relation with the duration of APD treatment. In particular, in patients on long-term APD, we observed an increase of intelectin-1, and a decrease of gelsolin.

These changes were also observed by in vitro treatment of mesothelial cells with oxidative or pro-fibrotic stimulus which supported the biological role of these proteins' changes.

In order to clarify the biological meaning of the observed differences, further step of our study will consist of the longitudinal evaluation of PDE proteome.

Biological significance

The in-depth proteomic characterization of peritoneal dialysis effluent (PDE) in pediatric patients by the combined use of Combinatorial Peptide Ligand Library (CPLL) and two dimensional electrophoresis allowed to detect 1788 spots, a relevant part (724) of which were previously undetected in sample untreated with CPLL. In patients on long-term automated peritoneal dialysis, this proteomic approach allowed to identify 29 potential

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biomarkers that could be of help to identify patients with subclinical inflammation and/or developing peritoneal membrane fibrosis, thus adapting dialysis treatment accordingly.

1. Introduction

The identification of clinically relevant biomarkers represents a major challenge in clinical proteomic. In this context, biological fluids are interesting materials because their collection is less invasive compared with obtaining tissue biopsies. Moreover, the fluids that are produced by, or are in close proximity to, a diseased organ may increase the probability of finding biomarkers of organ function or damage.

Peritoneal dialysis (PD) represents the dialysis modality of choice for pediatric patients with end-stage renal disease (ESRD) while awaiting kidney transplantation. In growing children, adequate metabolic control and acid-base balance maintenance are even more important than in adults [1]. Furthermore, the preservation of peritoneal membrane (PM) integrity and function is of major importance in view of renal replacement therapy required for many decades. Deleterious effects of PD solutions have been long debated, and a series of studies raised major clinical concerns regarding the prolonged exposure of the PM to standard PD solutions with high glucose and lactate concentrations, low pH, high osmolarity, and high levels of glucose degradation products (GDPs). These concerns are substantiated by adult peritoneal biopsy data [2] and pediatric data on deterioration of PM transport function with time even in the absence of peritonitis episodes [3]. In automated peritoneal dialysis (APD), the modality usually prescribed in pediatric patients, the PD solution is frequently exchanged over the course of a dialysis session, and such treatment regimens further increase the significance of PD fluid biocompatibility. In patients treated with APD for many years, these structural alterations contribute to the development of fibrosis of the connective tissue underlying the mesothelial cell layer which may develop to sclerosis of PM and loss of solute transport and ultrafiltration (UF) capacity [4].

Peritoneal dialysis effluent (PDE) is drained from the peritoneal cavity at the end of each dwell of an APD session and contains plasma proteins which crossed the PM by means of a diffusion mechanism dependent on molecular size and weight, as well as some proteins of mesothelial cell origin. For this reason, PDE represents an attractive biochemical window into the peritoneum and a useful reservoir of potential clinical biomarkers that may help to predict individual risk of developing PM sclerosis.

In this study we performed a proteomic characterization of PDE samples collected in patients with different APD treatment duration by the combined use of Combinatorial Peptide Ligand Library (CPLL) technology and two dimensional electrophoresis (2-DE). CPLL has been recently described as an efficient approach to decrease the concentration of dynamic range of complex protein mixtures and at the same time increase the capture of previously undetected proteins [5,6]. Each CPLL bead contains a unique hexapeptide ligand which can theoretically interact with one or a few proteins. Once the most abundant protein species have saturated their binding sites, the remaining molecules are washed away, whereas low abundant protein species are progressively enriched [7,8]. In order to optimize the compatibility between CPLL treated PDE samples, we evaluated several cleaning precipitation methods. These experiments allowed to increase both the performance of 2-DE and the enhancement of detected proteins.

Using the PDE of 19 pediatric patients with different primary renal diseases and at different time of APD, this proteomic strategy, paired with 2-DE analysis, aimed to the detection of new low abundance proteins and the identification of potential biomarkers of PM damage was associated with long-term APD treatment. Moreover, we tried to explain the biological role of the identified proteins by means of in vitro experiments.

2. Materials and methods

All chemical reagents were analytical grade (Fluka, Buchs, CH). Solutions were freshly prepared using bi-distilled Milli-Q water. The protein amounts in each experiments were measured using Bradford assay (BioRad).

2.1. Patients

We studied 19 patients with a median age of 3.9 years (range 0.2–16.6), weight of 14.5 Kg (range 2.8–49.6), and height of 92 cm (range 48–172). Median patients' body surface area (BSA), calculated by means of the Haycock formula [9], was 0.59 m^2 (0.18–1.53). Patients' primary renal disease was renal dysplasia in 6 cases, nephronophthisis in 5 cases, hemolytic uremic syndrome, focal segmental glomerulosclerosis, membranoproliferative glomerulonephritis, renal cortical necrosis, congenital nephrotic syndrome, autosomal recessive polycystic kidney disease, diffuse mesangial sclerosis and autosomal recessive polycystic kidney disease in 1 case each (Table 1). At the moment of the study, patients had been on APD with glucose-based solutions for a median of 13 months (range 1–38 months) and had not suffered from any infection episode for at least 1 month. For each patient a single time sample was collected. The set of 19 patients covers a time range of APD treatment from one to thirty-eight months. Written, informed consent was obtained from patients and/or their parents.

2.2. Sample preparation

PDE samples were taken at the end of each dwell, when the patient's abdomen was accurately drained and dialysate volume was measured. Immediately after the collection, the samples were centrifuged at 2000 ×*g* for 10′ at 4 °C to remove cells and debrides. The supernatant was dialysed overnight at 4 °C against 25 mM phosphate buffer pH 7.4 (binding buffer). Protease inhibitor cocktail was added to the protein amounts obtained from dialysed PDE samples and stored at −80 °C until used.