Neutral pH and low–glucose degradation product dialysis fluids induce major early alterations of the peritoneal membrane in children on peritoneal dialysis

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The effect of peritoneal dialysates with low–glucose degradation products on peritoneal membrane morphology is largely unknown, with functional relevancy predominantly derived from experimental studies. To investigate this, we performed automated quantitative histomorphometry and molecular analyses on 256 standardized peritoneal and 172 omental specimens from 56 children with normal renal function, 90 children with end-stage kidney disease at time of catheter insertion, and 82 children undergoing peritoneal dialysis using dialysates with low–glucose degradation products. Follow-up biopsies were obtained from 24 children after a median peritoneal dialysis of 13 months. Prior to dialysis, mild parietal peritoneal inflammation, epithelial-mesenchymal transition and vasculopathy were present. After up to six and 12 months of peritoneal dialysis, blood microvessel density was 110 and 93% higher, endothelial surface area per peritoneal volume 137 and 95% greater, and submesothelial thickness 23 and 58% greater, respectively. Subsequent peritoneal changes were less pronounced. Mesothelial cell coverage was lower and vasculopathy advanced, whereas lymphatic vessel density was unchanged. Morphological changes were accompanied by early fibroblast activation, leukocyte and macrophage infiltration, diffuse podoplanin presence, epithelial mesenchymal transdifferentiation, and by increased proangiogenic and profibrotic cytokine abundance. These transformative changes were confirmed by intraindividual

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comparisons. Peritoneal microvascular density correlated with peritoneal small-molecular transport function by uni- and multivariate analysis. Thus, in children on peritoneal dialysis neutral pH dialysates containing low–glucose degradation products induce early peritoneal inflammation, fibroblast activation, epithelial-mesenchymal transition and marked angiogenesis, which determines the PD membrane transport function.

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Peritoneal dialysis (PD) is the preferred dialysis modality in young children and is increasingly applied in adults.[1] It provides a cost-effective renal replacement therapy independent of a vascular access, greater individual freedom, and at least equal patient outcome within the first years of treatment compared with hemodialysis.[2] A major drawback of PD is the toxicity of conventional dialysis solutions, which expose the peritoneum to high concentrations of glucose, glucose degradation products (GDP), lactate, and an acidic pH. In adults on chronic PD, these components have been associated with progressive peritoneal mesothelial cell loss, submesothelial fibrosis, and vasculopathy. At the time of PD failure, submesothelial blood and lymphatic vessel number are increased.[3,4] A gradual increase in small-solute transport rates and loss of ultrafiltration occur with extended PD,[5] particularly when increasing concentrations of glucose are applied.[6] According to animal and mathematical models, ultrafiltration and solute transport are mainly defined by ultrasmall pores (i.e., aquaporin-1) and small pores present in peritoneal vessels.[7–9] The interstitial space is not initially a relevant barrier to water and solute transport, but eventually, if major fibrosis occurs with time on PD, osmotic conductance is reduced and contributes to impaired ultrafiltration.[10] Human data on the relationship between peritoneal membrane ultrastructure and membrane transport function are, however, scant.

In second-generation double-chamber PD solutions, glucose is stored at very low pH separated from the lactate or bicarbonate buffer and electrolytes. This largely prevents GDP formation during heating and storage. Prior to use, mixture of the compartments results in dialysis solution with a neutral pH. While randomized controlled trials have demonstrated better preservation of residual renal function[11–13] and suggest reduced peritonitis incidence and severity[14] with the use of second-generation PD fluids, their effects on peritoneal membrane transport function are uncertain. Higher small-molecule transport and lower ultrafiltration rates were found with neutral-pH, low-GDP fluids compared with conventional solutions during the first 6 treatment months, but the difference vanished with time on PD.[13,15] The histomorphological changes associated with low-GDP fluid usage in humans are unknown, and the molecular events underlying PD-induced membrane transformation have mainly been described in animals and *in vitro* to date.[16–18]

In this work we performed a comprehensive analysis of peritoneal tissue biopsies from children with end-stage kidney disease prior to and during maintenance PD with low-GDP fluids, in comparison with healthy children matched for age and gender. The pediatric population appeared of particular interest because the absence of aging and lifestyle-related tissue and vascular pathology in this age group should allow a highly sensitive analysis of the effects of chronic kidney disease (CKD) and PD on peritoneal membrane integrity and function.

RESULTS

The parietal peritoneal membrane in CKD5

The patient and parietal tissue selection procedure and the clinical and biochemical findings of the 3 patient cohorts are described in Figure 1 and Table 1. Biochemical findings reflect renal function–related changes, but without differences between CKD5 and PD patient groups. Mesothelial cell coverage and submesothelial thickness were similar in children with CKD5 and controls. CD31-positive submesothelial microvessel density and microvessel number per mm section length were 40% and 20% higher in CKD5 patients, due to an increase in blood microvessel density (Table 2). Blood microvessel density negatively correlated with age in controls (rho = –0.40, $P = 0.003$) but not in CKD5 (rho = 0.15, $P = 0.164$). The ratio of vessel lumen to total vessel diameter (L/V) of peritoneal arterioles, a marker of vasculopathy, was lower in CKD5, and endothelial telomeres were relatively shorter (Table 2). Fibrin deposits, CD45-positive leukocytes, and epithelial-to-mesenchymal transition (EMT) cells were more prevalent in CKD5 patients, and transforming growth factor-ß (TGF-ß)-induced pSMAD was increased 4-fold.

Peritoneal membrane transformation with low-GDP PD

In children on PD, daily body surface area–adjusted dialytic glucose exposure increased with PD vintage (Table 3). Peritoneal mesothelial coverage was lower with longer PD vintage, with 60% of patients having a largely denudated peritoneal surface after more than 2 years of PD (Table 3). Submesothelial thickness was 23% and 58% higher after up to 6 and 12 months of PD compared with CKD5 and again higher beyond 4 years of PD. In a multivariate analysis including age, previous peritonitis, dialytic glucose exposure, PD duration, and EMT presence, the latter 2 were independently associated with submesothelial thickness (Supplementary Table S1A). Submesothelial thickness was increased by 20% per year of PD, and EMT presence predicted a 125-µm greater submesothelial thickness (both $P < 0.001$).

CD31-positive total microvessel density was 44% and 90% higher after a median of 4 and 9 months of PD, respectively, and not further increased in children with longer PD