Effect of peritoneal dialysis on renal morphology and function

Andrzej Bręborowicz¹, Krzysztof Pawlaczyk¹, Alicja Połubinska¹, Kinga Górna¹, Anders Wieslander², Ola Carlsson², Paul Tam³ and George Wu³

¹Department of Pathophysiology, Poznan Medical School, Poland, ²Gambro AB, Lund, Sweden and ³Institute of Kidney Lifescience Technologies, Toronto, Canada

Abstract

Background. Results of clinical studies suggest that peritoneal dialysis (PD) is less harmful to the residual renal function than haemodialysis. However, we have no objective data describing the potential injuring effect of PD to kidney. We studied in rats after unilateral nephrectomy changes in renal structure and function after 12 weeks exposure to standard, glucose-based PD fluid.

Methods. One month after removing one kidney PD catheters were implanted in rats and during the following 12 weeks, twice a day, animals were infused with 20 ml of 3.9% glucose dialysis fluid containing high concentration of glucose degradation products. Rats not infused with the dialysis fluid served as control (CON). At the beginning and after 12 weeks of the study renal creatinine clearance, urinary excretion of albumin, N-acetyl-β-glucosaminidase (NAG) and cytokines were measured. Concentration of malondialdehyde (MDA), advanced glycation end products (AGEs) and monocyte chemoattractant protein-1 (MCP-1) were measured in serum samples. Morphology of the kidneys was evaluated in the light microscope.

Results. After 12 weeks exposure to the dialysis fluid serum MDA, AGEs and MCP levels were increased as compared with CON by 80%, \( P < 0.002 \), 29%, \( P < 0.05 \) and 71%, \( P < 0.005 \), respectively. Renal clearance of creatinine was comparable in both groups, but urinary excretion of albumin was increased by 55% in control group and by 160% in the studied group, \( P < 0.001 \); whereas urinary excretion of NAG was not changed in control group but increased by 125% in the studied group, \( P < 0.01 \). Increase of the remnant kidney's weight was higher (77%, \( P < 0.01 \)) in the CON group, but accumulation of the extramesangial matrix in glomeruli and collagen in the peritubular space was stronger in the studied group by 69%, \( P < 0.0001 \) and 274%, \( P < 0.0001 \), respectively.

Conclusion. Chronic exposure of rats to the glucose-based dialysis fluid causes morphological changes in the renal glomeruli similar to diabetic nephropathy. Albuminuria increases what may accelerate progression of the kidney damage.

Keywords: glucose; peritoneal dialysis; renal infection; renal morphology

Introduction

Preservation of the renal residual function is one of the most important factors determining adequacy of the peritoneal dialysis (PD). Diaz-Buxo et al. [1] reported that survival of patients treated with PD was strongly correlated with the residual renal function but not with the peritoneal clearance. Patients treated with PD have lower risk of the residual renal function loss than those on haemodialysis [2]. Decline of the residual renal function is a multifactorial process. However, one can assume that the process of PD may also contribute to that pathology.

Glucose, which is used as an osmotic agent in majority of the PD fluid is absorbed from the dialysate in large quantities (100–250 g/day). Such daily systemic load of glucose may lead to metabolic disorders, and changes in structure of various organs similar to pathologies observed during diabetes mellitus. Concentration of carbonyl compounds in the body fluids is increased during uraemia which results in the modification by these compounds of the proteins and lipids’ structure and function [3]. In patients with end-stage renal failure and treated with PD, carbonyl compounds such as glucose degradation products (GPDs) which are generated during autoclaving of the glucose solutions are absorbed also from the dialysate and instilled into the abdominal cavity [4]. Accelerated formation of the advanced glycation or lipoxidation end products in the presence of GPDs...
may result in enhanced oxidative stress [5] and inflammation [6]. Methylglyoxal being one of many GDPs induces apoptosis in rat mesangial cells, which may be a possible mechanism for the loss of glomerular cells in various types of glomerulosclerosis [7]. Oral administration of methylglyoxal in mouse leads to fibrosis of kidney with thickening of the glomerular basement membrane [8].

In patients with end-stage renal failure, progressive decline of the renal function is a multifactorial process caused by the primary pathology leading to renal failure; uraemia being a consequence of the renal failure and dialysis itself due to its proinflammatory action. It is difficult in a clinical environment to distinguish how important is each of the above listed factors and how we can prevent progression of that process. At the same time, it is known that total loss of the renal function dramatically reduces adequacy of the PD [9].

We present results of the *in vivo* experiments performed on rats after unilateral nephrectomy, which during 12 weeks were infused intraperitoneally with the glucose-based dialysis fluid, rich in GDPs, which was absorbed from the abdominal cavity. Application of such experimental model allowed us to expose animals to a large dose of potentially toxic components of the dialysis fluid during a relatively short period of time as compared with clinical setting. Changes in the renal morphology and function were evaluated in such animals and compared with rats that were not exposed to dialysis fluid.

**Material and methods**

Study was done on male Wistar rats. All the experiments were performed according to protocol approved by Animal Ethics Committee of the authors’ institution.

**Description of the experiment**

Experiment was performed on 18 male Wistar rats with mean body weight 345 ± 18 g (range 316–380 g). At the beginning of the study all animals were placed for 24 h into the metabolic cages to measure urine output, glomerular filtration rate (GFR) was evaluated with creatinine clearance, and excretion of albumin. Blood sample was drawn, under ether anaesthesia, from the tail vein at the end of the 24-h stay in the metabolic cage.

After completing the first metabolic evaluation of the renal function, left kidney was removed in all animals under ether anaesthesia supplemented with neuroleptanalgesia (fentanyl 0.004 mg/100 g bw and droperidol 0.2 mg/100 g bw). Afterwards animals were placed into the individual cages to heal after surgery for 1 month. At the end of that period, metabolic evaluation of the renal function was performed as done previously. Additionally urinary excretion of N-acetyl-β-D-glucosaminidase (NAG), transforming growth factor-β (TGFβ) and tumour necrosis factor-α (TNFα) was measured. Concentration of the advanced glycation end products (AGES) malondialdehyde (MDA), monocyte chemoattractant protein-1 (MCP-1) and TNFα were measured in serum samples.

Then, peritoneal catheters were implanted in 12 rats according to the technique elaborated in our lab, and six rats were left as control (CON). During the following 12 weeks rats with implanted peritoneal catheters (PD group) were infused (20 ml) twice a day with the standard dialysis fluid Gambrosol-Trio (Gambro, Lund, Sweden) which has the following composition: sodium 131 mmol/l; calcium 1.70 mmol/l; magnesium 0.24 mmol/l; chloride 96 mmol/l; lactate 39 mmol/l; glucose 39 g/l that additionally was autoclaved to generate GDPs. Such treatment caused an increase of GDPs concentration in the solution as reflected by higher absorbance at the range of the wavelength 230–280 nm, when compared with the original Gambrosol-Trio fluid (Figure 1).

In additional experiments, we found that the implantation of the peritoneal catheters that were left in the abdominal cavity the following 12 weeks, but not infused with any dialysis solution, does not cause any morphological changes in the peritoneum or kidneys as compared with control animals in which peritoneal catheters were not implanted. Peritoneal permeability to glucose and total protein was identical in both groups during 90 min peritoneal equilibration test (PET) performed with Gambrosol-Trio4. Renal function measured with such parameters as urine output, glomerular creatinine clearance and albuminuria were also comparable in both groups of animals.

Infused dialysis fluid was allowed to absorb from the peritoneal cavity. At weekly intervals, dialysate was drained after 4 h dwell for the measurement of the cell count and differential count to exclude peritonitis. In no animal peritonitis was detected during the whole experiment. Rats from both experimental groups were kept in the individual cages. All animals completed the study. After 12 weeks, in all animals, metabolic evaluation of the renal function was performed according to identical protocol as at the beginning of the study. Rats were afterwards sacrificed and the remaining kidney was removed for examination in light microscopy as aforementioned.

**Fig. 1.** Absorbance measured at the range of the wavelength 230–280 nm of the Gambrosol-Trio fluid and of the same solution but additionally autoclaved (high GDP fluid).
Morphological study

Excised kidneys were fixed in phosphate-buffered 4% formalin (pH 7.4) for morphological evaluation in light microscopy, followed by staining with periodic acid/Schiff reagent (PAS) for the presence of mesangial extracellular matrix and with Masson trichrome for the visualization of collagen. Area of the cross section of 50 glomeruli was measured in each renal tissue slide using the software for digital analysis of the image (Lucia Screen Measurement 4.21, Laboratory Imaging Ltd, Czech Republic) mounted on the light microscope (Eclipse E400, Nikon, Japan). Change in the mean area size of the glomeruli in kidney removed at the beginning of the study, and in the remnant kidney removed from the same animal after 12 weeks of the experiment was calculated.

Biochemical analysis

Creatinine concentration in serum and urine samples was measured with colorimetric Jaffe method, available in a kit from Analco (Warsaw, Poland). Albumin concentration in urine was measured with ELISA for rat albumin available from Bethyl Laboratories (Montgomery, TX, USA). Activity of NAG in urine was measured with a colorimetric assay available from Roche Diagnostics Systems (Warsaw, Poland). In the presence of NAG, 3-cresolsulfophthaleinyl-N-acetyl-β-D-glucosaminide sodium salt is hydrolysed with the release of 3-cresolsulfophthalein Na salt that is measured photometrically at 580 nm. TGFβ concentration in urine was measured with ELISA available from Promega Corporation (Madison, WI, USA). TNFα concentration in urine and serum was measured with commercially available ELISA kit from Biosource International (Camarillo, CA, USA). MDA concentration in serum samples was measured with Lipid Peroxidation Assay Kit from Calbiochem (San Diego, CA, USA). MCP-1 concentration in serum samples was measured with ELISA kit available from Biosource International (Camarillo, CA, USA). Concentration of AGEs in the serum of the studied animals was assayed indirectly by the measurement intensity of fluorescence with excitation and emission wavelengths set at 355 and 460 nm, respectively.

Statistical analysis

Results are presented as mean values±SD. Analysis of the data was performed with Mann–Whitney or Wilcoxon test. A P-value <0.05 was considered significant.

Results

Four weeks after unilateral nephrectomy, we observed reduction of the renal creatinine clearance in all rats and an increase of the serum creatinine concentration (Table 1). Urine volume and albuminurina increased significantly (Table 1).

All animals completed the following 12 weeks of the study. During that time, rats gained weight mean by 84±25 g in the CON group and by 79±11 g in the studied group. Chronic exposure of the animals to the dialysis fluid resulted in the systemic oxidative stress
as reflected by increased serum level of MDA in the studied group (Figure 2A). In these animals we also found at the end of the study increased serum level of MCP-1 and AGEs as compared with CON group (Figure 2B and C). Serum concentration of TNFα had not increased during the study in either group.

After 12 weeks of the study we observed an increase in GFR in both groups; by 0.46 ± 0.77 µl/min in control and by 0.43 ± 0.78 µl/min in PD group. Albuminuria had increased during 12 weeks in all animals and that effect was stronger in PD group: by 1398 ± 738 vs 342 ± 572 µg/24 h in CON group, P < 0.01. Urinary excretion of NAG was comparable in both groups at the beginning of the study: 88.6 ± 41.5 units/24 h in CON group and 107.1 ± 26.1 units/24 h in PD group. However at the end of the experiment urinary excretion of NAG was significantly higher in PD group: 144.4 ± 25.4 units/24 h than in CON group: 102.6 ± 34.0 units/24 h, P < 0.01. At the same time urinary excretion of TNFα tended to be higher in PD group: 0.89 ± 0.51 pg/µmol creatinine vs 0.50 ± 0.38 pg/µmol creatinine in CON group, P = 0.067. The same trend was observed for urinary excretion of TGFβ: 229.6 ± 138.6 pg/µmol creatinine in PD group vs 123.4 ± 79.3 pg/µmol creatinine.

At the end of the study, rats were sacrificed and the remaining right kidney was removed and weighed. Weight of the right kidney was compared with the left one. In both groups absolute weight of the right kidney was higher as compared with the left one; in CON group by 0.83 ± 0.23 g and in PD group by 0.47 ± 0.21 g (P < 0.01). The differences became even more significant when the change in the kidney weight was corrected for the actual body size; in the CON group increase of the kidney weight was proportionally higher than increase of the rats’ body weight, whereas the opposite effect was seen in the PD group (Figure 3A). In both groups, size of glomeruli in the remnant kidney was increased as compared with the size of the glomeruli in the left kidney that was removed at the beginning of the study; in CON group 130.1 ± 56.6% of the glomeruli size in the left kidney and in PD group the respective value was 135.9 ± 124.7%. However in the PD group the amount of the PAS-positive substance in the glomeruli and the amount of collagen in the peritubular area were higher than in the CON group, respectively by 69% (P < 0.0001) and by 274% (P < 0.0001) (Figure 3B and C).

Discussion

Results of the experiments, which are presented in this article allowed us to evaluate the effect of chronic exposure to PD solution on the renal morphology and function. Rats were not undergoing a typical PD, because the instilled dialysis fluid was allowed to absorb from the peritoneal cavity. Such infusion model of chronic PD is used in all in vivo experiments on animals. Total absorption of the dialysate results in increased systemic exposure to the dialysate components, which may result in much stronger potential toxicity of these compounds than in patients treated with chronic PD. However, the amount of glucose absorbed daily into the systemic circulation of each rat was about 3 g/kg bw, which is comparable with daily glucose load in CAPD patients. On the other hand, animals were exposed to the dialysis fluid only for 12 weeks which is a much shorter period of time than treatment of uraemic patients with chronic PD, which lasts for years. Therefore, we think that the observations derived from our study may reflect, at least partially, the systemic and renal changes induced in dialysis patients exposed chronically to glucose-based...
dialysis solutions. In all animals, one kidney was removed at the beginning of the study to induce hypertrophy in the remaining one; effect similar to the changes in structure of the surviving nephrons in kidneys progressively damaged by any pathological process. At the same time, in the studied animals uraemia was not present as the changes in serum creatinine and GFR were negligible. We also found that the intra-abdominal presence of the implanted peritoneal catheter had no effect on structure and function of the peritoneum or kidneys. Therefore, the only factor which could contribute to observed changes in structure and function of the remnant kidney was chronic exposure to the dialysis fluid instilled intraperitoneally twice a day.

Infused dialysis fluid was totally absorbed from the abdominal cavity, which resulted in the systemic exposure of animals to large dose of glucose, GDPs, lactate, and other potentially harmful substances present in the dialysate. We found that such treatment results in the induction of systemic inflammation (Figure 2). Both glucose and its degradation products contribute to the oxidative stress [10]. In our experiments, rats chronically exposed to glucose-based dialysis fluid had increased concentration of serum MDA (Figure 2A). In diabetic nephropathy increased presence of MDA was also detected in glomeruli [11]. Reactive carbonyl compounds originating from the dialysis fluid and oxidative stress enhance formation of AGEs [12], which may explain increased serum AGEs levels in serum of rats exposed to the dialysis fluid (Figure 2C). Increased level of AGEs and the oxidative stress in studied rats could explain enhanced concentration of chemokine MCP-1 in serum (Figure 2B). Despite unchanged serum TNFα level, we observed a tendency for increased urinary excretion of that cytokine in rats treated with the dialysis fluid. Therefore we speculate that TNFα present in urine originated mainly from the mesangial cells. AGEs increase expression of TNFα in the glomeruli of rats with diabetes [13]. Increased urinary and renal interstitial TNFα level was observed in diabetic rats [14]. We believe that prolonged, exceeding 12 weeks, exposure of the kidney to the inflammatory milieu may accelerate decline of its function as it was suggested based on the results from the clinical studies [15].

Removal of one kidney leads to hypertrophy of the remnant one. Indeed in control rats increase of the remnant kidney weight was proportionally higher than change in their body weight during the observation period (Figure 3A). However in animals exposed to the dialysis fluid the remnant kidney grew less than the whole body. The only possible explanation of that finding might be dialysis-induced inflammation and direct damage to the remnant kidney by high glucose load, GDPs or other components of the dialysis fluid. Despite lack of the significant increase in the urinary excretion of TGFβ in rats exposed to the dialysis fluid, we observed increased positive staining for collagen in the peritubular interstitial area in the remnant kidney removed from these animals at the end of the study (Figure 3C). High glucose stimulates renal synthesis of collagen via TGFβ dependent pathway [16], but that process also may be stimulated via mechanisms independent of that cytokine [17]. Increased accumulation of the PAS-positive material in the glomeruli of rats treated with glucose-based dialysis fluid (Figure 3B) is reflecting accumulation of the extracellular mesangial matrix, which is a primary event leading to glomerulosclerosis during diabetic nephropathy.

Despite pathological changes in the morphology of the kidney from rats exposed chronically to the dialysis fluid, we did not find any significant decline in renal clearance of creatinine. That discrepancy could be due to chronic expansion of their extracellular space with absorbed fluid, which increases glomerular filtration [18]. However albuminuria was significantly higher in the rats treated with the dialysis fluid, which could be the first functional feature reflecting enhanced damage to nephrons in the remnant kidney. Higher urinary excretion of NAG in that group of animals may be a consequence of increased lysosomal turnover in epithelial cells of the proximal tubules reabsorbing a large amount of albumin [19]. However we cannot exclude that filtered GDPs caused direct injury to these epithelial cells [20].

Presented results demonstrate clearly that PD may contribute to kidney damage. High glucose load and GDPs are possible injuring factors. Recently introduced dialysis fluids with low concentration of GDPs will be probably not only less harmful to the peritoneum but also to the kidney. However, search for an alternative to glucose osmotic solute remains an important goal on our road towards more biocompatible dialysis fluids. Dialysis-induced systemic inflammation might also contribute to kidney damage. Therefore, reduction of the intraperitoneal and systemic inflammatory reaction should be also considered as an approach to slow down the decline of the residual renal function.

Conflict of interest statement. None declared.

References

are increased in serum of uremic patients: evidence for increased oxidative stress in uremia. FEBS Lett 1998; 427: 381–385

Received for publication: 17.11.05
Accepted in revised form: 12.6.06