Multidirectional approach to study peritoneal dialysis fluid biocompatibility in a chronic peritoneal dialysis model in the rat

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Abstract
Background. Peritoneal dialysis causes the functional and morphological changes in the peritoneum that result from the bioincompatibility of dialysis solutions. We present a model of chronic peritoneal dialysis in the rat that can be used for testing the biocompatibility of dialysis fluids.

Methods and Results. Long-term exposure of the peritoneum to dialysis solutions can be performed in rats with implanted peritoneal catheters. Sampling of the dialysate allows the evaluation of intraperitoneal inflammation by examining cell differential and dialysate cytokine levels. Peritoneal permeability can be evaluated at designed time intervals with the peritoneal equilibration test (PET). At the end of dialysis, peritoneal histology is studied with light and electron microscopy.

Conclusions. Such a multidirectional approach is an effective way to test biocompatibility of dialysis solutions.

Keywords: inflammatory reaction; morphology; peritoneal dialysis; peritoneal transport; rat model

Introduction

Peritoneal dialysis fluids and the peritoneal dialysis procedure itself are not physiological. The biocompatibility of peritoneal dialysis solutions is being tested in experimental animals in several laboratories around the world. There is still, however, no agreement on how this should be performed. Various authors use different laboratory animals and estimate different parameters after different peritoneal exposure times to dialysis fluids.

It is known that functional and morphological changes observed in dialysed patients become more severe with time on dialysis. It thus seems obvious that for the testing of biocompatibility of peritoneal dialysis solutions in an experimental animal, a chronic model must be developed. At present, it is unclear how changes observed following an infusion of dialysis solution relate to chronic exposure. Additionally, the experimental model should allow dialysate sampling which can provide important information on intraperitoneal inflammation and peritoneal function during the time of dialysis. Only an animal with a chronic catheter makes it possible to compare the same parameters on the same animal at different time points.

Here we present a model of chronic peritoneal dialysis in the rat, which allows for estimation of functional and morphological changes in the peritoneum of dialysed rats.

Model description—method and results

All experiments are done on male Wistar rats weighing 300–350 g. After omentectomy, catheter implantation is performed as previously described [1].

After instillation of 20 ml of fluid, intraperitoneal inflammation is measured by sampling the effluent dialysate after 4 h of dwell. In rats (n=8) exposed to Dianal®, 3.86% (Baxter, Deerfield, USA), the intensity of the inflammatory reaction is the highest just after catheter insertion and decreases with time. After 6 days of dialysis, the number of peritoneal cells in the dialysate was significantly lower when compared with the first exchange, performed 24 h after catheter implantation (day 6: 996 ± 464 cells/µl; day 1: 5754 ± 3962 cells/µl; P < 0.001). The macrophage/neutrophil ratio expressed as a percentage was higher on day 6 compared with day 1, reflecting an increase in the number of macrophages with a parallel decrease in the number of neutrophils in cell differential (day 6, 208 ± 55%; day 1, 96 ± 22%; P < 0.01). The dialysate level of monocyte chemoattractant protein-1 (MCP-1)
was lower on day 6 when compared with day 1 (day 6, 6.3 ± 1.7 pg/ml; 19.6 ± 9.9 pg/ml; \( P < 0.001 \)).

Peritoneal transport was measured with the peritoneal equilibration test (PET) performed with 30 ml of Dianel 3.86%. The PET is always done at the beginning of the experiment as baseline (PET 1) and repeated at the end of the study period (PET 2). The details of the PET procedure have been presented previously [1]. The comparison of PETs performed in rats dialysed for 6 weeks with Dianel 2.27% (n = 8) showed higher drained volume during PET 2 in comparison with PET 1 (30.5 ± 2.2 ml vs 26.2 ± 2.6 ml; \( P < 0.05 \)). During PET 2, the D/Do ratio of glucose was higher than in PET 1 (4 h samples, 0.09 ± 0.01 vs 0.06 ± 0.01; \( P < 0.02 \)) and the D/Do ratio of total protein was also lower (0.050 ± 0.007 vs 0.063 ± 0.014; \( P < 0.05 \)). Thus, high peritoneal permeability at the beginning of the study possibly can be explained by activation of the inflammation, due to the surgical procedure during omentectomy and insertion of the catheter.

Morphological changes in the peritoneum were estimated in peritoneal biopsies taken from each animal at the end of the study. In light microscopy of samples taken from peritoneum lining the surface of the liver of chronically dialysed rats, the thickening of the submesothelial tissue can be studied. It is due to an increased amount of collagen fibres, oedema and an increased number of submesothelial cells. The measured thickness of the peritoneum from rats exposed to dialysis was significantly greater than in control animals not exposed to dialysis solution (38.07 ± 40.10 lμm vs 3.10 ± 0.79 lμm; \( P < 0.02 \)). Regarding immunohistochemistry, the accumulation of collagen I and III and advanced glycation end-products was observed. In electron microscopy, the disintegration of collagen fibres and activation of submesothelial macrophages (increased phagosome content) as well as fibroblasts (numerous extensive processes) are also found.

Discussion

The technique of implantation of a chronic double-cuff catheter in the rat, originally presented by Moore [2], was modified in our laboratory. Omentectomy is always performed during catheter insertion as, in our experience, the omentum may block the catheter and prevent drainage of the dialysate. A similar problem was reported by Moncrieff et al. during dialysis in the dog [3]. In our experimental model, dialysis fluid is always supplemented with antibiotics (cefuroxim 50 mg/l, gentamycin 5 mg/l) to reduce the risk of peritonitis, which is high because all dialysis fluid exchanges are performed in awake rats. Despite extreme precautions taken to perform all procedures under sterile conditions, up to 10% of animals are lost due to peritonitis. Such animals are excluded from further study in order to minimize overlapping of the morphological and functional changes in peritoneum induced by peritonitis with those that result from the bioincompatibility of the tested dialysis fluid.

The PET is a reliable method for repeated evaluation of the peritoneal permeability during chronic dialysis. It was shown previously that PET performed twice in the same animal, on days 1 and 3 with a 1-day interval, is highly reproducible [4].

In conclusion, we believe that the presented rat model of chronic peritoneal dialysis is suitable to study the long-term effects of peritoneal dialysis solutions on the peritoneum. Evaluation of various functional and morphological parameters is required for complete evaluation of the dialysis fluid biocompatibility.

References