Ultrastructure of basement membranes of peritoneal capillaries in a chronic peritoneal infusion model in the rat

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Abstract

Background. Long-term peritoneal dialysis with glucose-based dialysis solutions has been associated with diabetiform alterations of peritoneal tissue. A peritoneal infusion model in the rat was developed to study the effect of chronic infusion of a glucose-based dialysis solution and an isotonic non-glucose solution on the ultrastructure of the basement membranes of peritoneal capillaries. The effect of ageing was also studied in an untreated control group.

Methods. A vascular access port (Rat-o-Port) with attached peritoneal catheter was implanted subcutaneously in the neck of nine male Wistar rats. The rats were divided randomly into three groups: the glucose group (n = 3) was infused daily for 20 weeks with 60 ml/kg body weight 3.86% glucose solution. A control group (n = 2) was infused daily for 20 weeks with 60 ml/kg body weight Ringer’s lactate. The untreated control group (n = 4) was studied at the onset of the experiment and after 20 weeks. Omental tissue was obtained from each rat at the end of the experimental period for ultrastructural examination.

Results. Extensive lamination of basement membranes of omental capillaries was found in the glucose group. This was in contrast to the untreated control group where clear, single basement membranes were seen at the onset of the experiment and after 20 weeks. These latter findings were similar to those in the Ringer’s lactate group.

Conclusions. The chronic infusion model in the rat is suitable for the investigation of the effects on the ultrastructure of peritoneal capillaries of chronic exposure to dialysis fluids. The duplications of basement membranes of omental capillaries found in the glucose group show a striking resemblance to those found in long-term peritoneal dialysis patients. This suggests a role for glucose in the development of peritoneal ultrastructural alterations found in long-term peritoneal dialysis.

Keywords: glucose dialysis solution; long-term peritoneal dialysis model; peritoneal capillary basement membrane; ultrastructure

Introduction

Long-term peritoneal dialysis with glucose-containing dialysis solutions has been associated with changes in peritoneal morphology [1,2] and function [3,4]. These include lamination of capillary basement membranes [5–7]. The effect of daily exposure to intraperitoneal fluid, and ageing or growth, on the development of these ultrastructural alterations of the peritoneal capillary basement membranes has not been elucidated.

A chronic peritoneal infusion model was developed in the rat to investigate the effect of infusion of a glucose-based dialysis solution and an isotonic non-glucose solution on the ultrastructure of the basement membranes of peritoneal capillaries. Furthermore, the effect of growth and ageing of the animals was studied in an untreated control group.

Methods

Animals

Nine male Wistar rats (Harlan CBP, Zeist, The Netherlands) with a mean body weight of 325 g (SD 24.2 g) were divided randomly into three groups: three rats in the glucose group, two in the Ringer’s lactate control group and four in the untreated control group. The first two groups were anaesthetized by intramuscular administration of a mixture of ketamine, xylazine and atropine (8 mg, 4 mg and 5 μg per 100 g body weight) when implanted with a Rat-o-Port (Access Technologies, Norfolk Medical, Skokie, IL). The Rat-o-Port was implanted subcutaneously in the neck, and the attached silicone catheter (lumen 1.1 mm,
Fig. 1. Transmission electron micrographs of omental capillaries. (A) Electron micrograph of an omental capillary (×11 600) obtained in a rat after 20 weeks of daily infusion with glucose-based dialysis solution. Marked lamination of the basement membrane was present (arrow). (B) Omental capillary (×15 800) of a rat in the untreated control group at the start of the experiment. A clear unilamellar basement membrane (arrow) of the peritoneal capillary was present. (C) Electron micrograph of an omental capillary (×15 800) obtained in a Ringer’s lactate rat after 20 weeks daily infusion. A single basement membrane was found (arrow).
length was adjusted per rat) with one dacron cuff was tunnelled subcutaneously into the peritoneal cavity. During the recovery period of 1 week, the catheter was infused daily with 1 ml of heparinized saline (5 IU/ml 0.9% NaCl).

The glucose group was infused daily for 20 weeks with heparinized (5 IU/ml) 3.86% glucose dialysate Diancel (Baxter Healthcare S.A., Castlebar, Ireland) 60 ml/kg body weight per day, and the rats in the Ringer’s lactate group with heparinized (5 IU/ml) Ringer’s lactate 60 ml/kg body weight per day. The maximum volume was set at 20 ml/day when the body weight exceeded 420 g. Two rats in the untreated control group were studied at the start of the experimental period and two rats after 20 weeks. Twenty weeks after the start of the daily intraperitoneal infusions, omentum specimens were obtained.

Electron microscopy

The tissue was fixed in 4% paraformaldehyde, followed by post-fixation in 1% osmium tetroxide, block-staining with 1% uranyl acetate, one-step dehydration in dimethoxypropane and embedding in epoxysin LX-112. EM sections were stained with tannic acid [8], uranyl acetate and lead citrate, and studied with a Philips CM 10 (Eindhoven, The Netherlands).

Results

Investigation with transmission electron microscopy of the basal membranes of omental capillaries of the glucose group revealed extensive lamination and reduplication after 20 weeks of infusion with glucose-based dialysis solution (Figure 1A). This finding was in contrast to the Ringer’s lactate group and the untreated control group. In the latter two groups, clear single basement laminas were observed. The appearance of the basement membranes at the onset of the experiment in the untreated control group (Figure 1B) was not different from the presentation after 20 weeks (data not shown). Daily infusion with Ringer’s lactate for 20 weeks did not cause alterations of the ultrastructural appearance of the basement membranes of the omental capillaries as unilamellar basement membranes were found (Figure 1C).

Discussion

A 20 week daily infusion with the hypertonic glucose-based dialysis solution caused marked lamination of the basement membranes of omental capillaries. During the same infusion period with the Ringer’s lactate solution, the ultrastructure did not change. Daily infusion was therefore not the trigger to induce alterations in the peritoneum. Neither did growth or ageing appear to influence peritoneal capillary ultrastructure as a clear single basement membrane was found after 20 weeks in the untreated control rats. The isotonic Ringer’s lactate solution contains the same constituents as the glucose-containing dialysis solution, but without the high glucose concentration, suggesting a role for the extremely high glucose concentrations. Hyperglycaemia-induced ultrastructural changes in diabetic microangiopathy [6,9] show marked parallels with those found in the peritoneal capillary basement membranes, emphasizing the potentially deleterious effects of continuous exposure to high glucose concentrations.

The lifetime of a rat is ~2.5 years. Twenty weeks infusion therefore represents ~15% of the life span.
Fifteen percent of a human life is ~ 10 years, assuming an average maximum age of 75 years. Ten years of peritoneal dialysis can be considered as long-term treatment. Therefore, the experimental period of 20 weeks can be taken as an approximation of long-term peritoneal dialysis.

The similarity of our results found in the rats infused with a glucose-based dialysis solution and the abnormalities described in chronic peritoneal dialysis patients [6,10] suggests that the model is suitable for the investigation of the effects of chronic exposure to dialysis fluids on the ultrastructure of peritoneal capillary basement membranes. The similar ultrastructural changes in the glucose dialysis group and their absence in the control groups suggest a role for the high glucose concentration of the dialysis solutions in the development of peritoneal ultrastructural alterations.

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References


