Understanding and preventing the peritoneal damage caused by conventional dialysis solutions
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Chapter 5

Effects of Inhibition of the Polyol Pathway during Chronic Peritoneal Exposure to a Dialysis Solution

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ABSTRACT

INTRODUCTION

Peritoneal dialysis with glucose and lactate containing dialysis solutions stimulates peritoneal angiogenesis and fibrosis. These serious side-effects can also be induced by chronic peritoneal exposure to dialysis solutions in non-uremic rats. The high glucose concentrations of the dialysis solutions may saturate physiological glucose metabolism pathways and instead stimulate the polyol pathway that has been described to damage nerves and vessels in diabetes mellitus. To investigate the role of the polyol pathway in the development of fibrosis and angiogenesis, during chronic peritoneal exposure, the rate-limiting aldose reductase activity in the polyol pathway was inhibited in a chronic peritoneal exposure model in the rat, in which different administration routes were compared.

EXPERIMENTAL PROCEDURES

Three groups of rats received daily i.p. infusion with lactate/glucose 3.86% containing dialysate via a peritoneal catheter with a subcutaneous puncture device, for 14 weeks: group 1 received only the dialysis solution, groups 2 and 3 received in addition zopolrestat, administered either orally (group 2), or intraperitoneally (group 3). After sacrifice, omental tissue was examined by histology for the presence of fibrosis (picro-sirius red) and the number of blood vessels (CD31).

RESULTS

Histology revealed significantly less picro-sirius red-positive tissue in perivascular areas of both experimental groups and also in submesothelial areas of the oral group in comparison to the control group. The numbers of CD31-positive vessels per field were significantly less in both groups treated with zopolrestat, compared to the infusion only group: group 2: 9 (7–12), group 3: 17 (13–38) compared to group 1: 37 (32–39), p<0.05.

CONCLUSION

Combination of peritoneal exposure to dialysis fluids and administration of zopolrestat, a newly developed inhibitor of aldose reductase activity, resulted in less fibrosis and less peritoneal vessels than exposure to dialysis fluids only, in a long-term exposure model in the rat. Inhibition of the polyol pathway may thus offer an important contribution to allow long-term continuation of peritoneal dialysis.
INTRODUCTION

Long-term treatment of end stage renal disease patients with peritoneal dialysis can result in severe abnormalities in the peritoneum that can hamper the continuation of treatment [1]. The extremely high concentrations of glucose and the glucose degradation products in the peritoneal dialysis fluids are thought to be of major importance in the development of these abnormalities. They may saturate physiological glucose metabolism pathways and stimulate the polyol pathway. The polyol pathway is one of the routes whereby glucose can cause damage [2]. The enzyme aldose reductase is necessary for the first step of this pathway. Inhibition of aldose reductase activity has been shown to delay the onset of diabetic cataract formation \textit{in vivo} [3] and also corrected motor and sensory nerve conduction velocities in diabetic rats [4,5]. The aim of the present study was to investigate the effects of zoopolrestat, a newly developed aldose reductase inhibitor (ARI), during chronic peritoneal exposure to dialysis fluid in a rat model.

MATERIALS AND METHODS

Seventeen male Wistar rats acclimatized for one week before insertion of a peritoneal catheter under anesthesia with a mixture of ketamine: xylazine: atropine (4:2:1). A titanium/silicone device (Rat-o-Port, MTINC, 7IS, Access Technologies, Norfolk Medical, Skokie, IL, USA) was attached to the catheter and implanted subcutaneously in the neck of the animals. Peritoneal healing was allowed for one week after the catheter insertion by daily infusion of 1 mL of heparinized saline (5 IU/mL NaCl 0.9%), after which the experimental period started. All rats received a daily infusion of 60 mL/kg body weight (BW) 3.86% glucose containing dialysis solution (Baxter, S.A., Castlebar, Ireland) via the Rat-o-Port. Group 1 (n=5) received only infusion, group 2 (n=6) was also treated with zoopolrestat in the chow (100 mg/kg BW), group 3 (n=6) received zoopolrestat dissolved in the daily infusion (50 mg/kg BW; this dose was tested in a pilot study and was well tolerated for 4 weeks). After the exposure period of 14 weeks, omental tissue was examined for the presence of fibrosis and the number of vessels. The study was performed in accordance with the regulations required by the local ethics committee for animal experimentation.

HISTOPATHOLOGY

Omental and parietal peritoneal tissue were obtained from each rat and were processed for light microscopy. The tissues were fixed in freshly prepared 4% paraformaldehyde. Paraffin-embedded tissue was serially sectioned at 5µm thickness, sections were stained with hematoxylin/eosin and picro-sirius red F3B (PSR), the latter providing a brick red staining of all fibrillar collagen. For visualization of vessels, adjacent sections of omentum were immunohistochemically stained for CD31 (CD31, dilution 1:800, DAKO, Denmark). The number of all CD31-positive vessels per microscopic field of peritoneal tissue section was counted using a 25x flat field objective and a x10
ocular (Leitz Dialux 20, Leica, Rijswijk, The Netherlands). Five non-overlapping fields from the upper left to the lower right were investigated throughout each specimen. The amount of fibrosis was measured by semi-quantitative scoring of the extent of PSR staining of omental sections as previously described [6]. Briefly, submesothelial (SM), perivascular (PV) and intersegmental (IS) areas of omental tissue were judged separately, using as grades 0=normal presence of fibrous tissue, 1=mildly increased presence, 2=moderate, and 3=severe. The semi-quantitative scoring was assessed by two observers, that were blinded for the identity of the individual specimens; inter-observer variability was less than 10%.

**Statistical Analysis**

Medians and interquartile ranges are given unless stated differently. Analysis of variance was performed where after Mann-Whitney U tests were done to investigate differences between groups.

**Results**

The animals were healthy during the experimental period. The results of the scoring of the amount of vessels and the amount of fibrosis are given in Table 1. Treatment with zopolrestat and infusion resulted in less peritoneal vessels compared to infusion only. Oral administration of zopolrestat resulted in a lower number of vessels, than observed after i.p. administration. The extent of fibrosis was less in perivascular areas for both zopolrestat treated groups compared to the fibrosis found in the control group, which received only infusion of dialysis solution; fibrosis was also less abundant in the submesothelial areas in tissues of rats that received dialysis solution only.

| Table 1: Quantification of numbers of vessels and amount of fibrosis in omentum of rats. Medians and interquartile ranges are given. Scoring of fibrosis was performed in submesothelial, perivascular and intersegmental areas. |
|----------------------|----------------------|----------------------|
|                      | Infusion only (n=5)  | Infusion+zopolrestat orally (n=6) | Infusion+zopolrestat i.p. (n=6) |
| Vessels/field        | 37 (32-39)           | 9 (7-12)*             | 17 (13-38)*            |
| Fibrosis             |                      |                      |                        |
| Submesothelial       | 2 (2-3)              | 1 (0.75-1.25)*       | 2 (0.75-2)             |
| Perivascular         | 3 (2-3)              | 1*                   | 1 (0.75-2)*            |
| Intersegmental       | 2 (1.5-2.5)          | 1 (0.75-1)           | 1 (0.75-1)             |

*p<0.05 compared to infusion only

The polyol pathway has been described extensively as one of the processes that are involved in diabetic vasculopathy and neuropathy [4,5]. It has been reported that the polyol pathway would account for 33% of glucose flux in the eye, in circumstances of a high plasma glucose
In this pathway of intracellular glucose metabolism, glucose is reduced to sorbitol by aldose reductase coupled with oxidation of NADPH to NADP+. Sorbitol is then oxidized to fructose by sorbitol dehydrogenase, coupled with reduction of NAD+ to NADH. The precise nature of the biochemical imbalance that mediates the polyol pathway-linked functional abnormalities has not yet been fully elucidated [8,9]. Possible mechanisms include osmotic stress due to intracellular accumulation of sorbitol [3], an increased NADH/NAD+ ratio leading to pseudohypoxia [10], and enhancement of the formation of AGEs by fructose [11].

Zopolrestat inhibits aldose reductase activity and thus the rate-limiting first step of the polyol pathway, in which sorbitol is formed from glucose. It is therefore not possible to distinguish between the relative importance of the above mentioned mechanisms. This would have acquired an additional study using inhibition of sorbitol dehydrogenase.

In vitro studies with aldose reductase inhibition reported that human renal proximal tubular cells incubated in 25 mM glucose showed accumulation of fibronectin, type IV collagen, TIMP-1 (tissue inhibitor metalloproteinase type 1) and TIMP-2. The increase in fibronectin was inhibited by the aldose reductase inhibitor sorbinil, which was alleviated again when the cultures were replenished with 1mM sorbitol [12]. Also, administration of aldose reductase inhibition reduced the erythrocyte levels of 3-DG (3-dideoxyglucosone) and AGEs in hemodialysis patients, and also the intracellular sorbitol levels were reduced. These studies confirm that 3-DG and AGEs can be produced via the polyol pathway [13]. Also, an increased NADH concentration, as occurs by stimulation of the polyol pathway, has been reported to result in accumulation of glyceraldehyde-3-phosphate, dihydroxyacetone phosphate and fructose-1,6-biphosphate. These are precursors of methylglyoxal, which is an important intermediate in the formation of AGEs [10]. A study with daily administration of zopolrestat for 1 year in diabetic patients showed that left ventricular abnormalities could be stabilized and partially reversed [14].

In the present study both oral and i.p. administration of zopolrestat in combination with peritoneal exposure to a dialysis fluid resulted in less peritoneal blood vessels and fibrosis. In this respect, oral administration was more effective than the intraperitoneal administration of zopolrestat. The cause for this difference in effectiveness is not known at present. In a previous pilot study we administered 3 dosages of zopolrestat (0.5, 5 and 50 mg/kg BW) intraperitoneally for 4 weeks. Since the animals tolerated all tested dosages well, we chose to administer the highest dose in the present study. It is speculative whether the beneficial effects of zopolrestat i.p. were counteracted by local effects of the solvent. The implication is that in future studies in PD patients, oral administration may be applied, which is of course most convenient.

In conclusion, we found that administration of zopolrestat, a newly developed inhibitor of aldose reductase activity, is effective in preventing the development of peritoneal fibrosis and angiogenesis in a long-term peritoneal exposure model in the rat. Inhibition of the polyol pathway may thus offer an important contribution to allow long-term continuation of peritoneal dialysis.
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