Understanding and preventing the peritoneal damage caused by conventional dialysis solutions
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Chapter 6

Lisinopril in a Chronic Peritoneal Exposure Model in the Rat

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ABSTRACT

OBJECTIVE

Angiotensin II stimulates TGF-β mediated expansion of extracellular matrix. TGF-β is involved in the pathogenesis of peritoneal fibrosis. The aim of the present study was to investigate the effect of angiotensin converting enzyme (ACE) inhibition on peritoneal morphology in a chronic peritoneal exposure model in the rat.

MATERIALS

Twenty-five Wistar rats were all chronically i.p. exposed to dialysis fluid containing 3.86% of glucose. Group 1 (n=7) also received lisinopril in drinking water (0.15mg/mL) and was compared to the control group, infusion only (n=6), both groups treated for 16 weeks. Group 3 received infusions and lisinopril for 20 weeks (n=7) and was compared to group 4 (n=5), that received infusion only for 20 weeks.

Parietal peritoneum and omentum were investigated for the deposition of fibrous tissue (Sirius red) and the number of blood vessels (v. Willebrand, αSMA).

RESULTS

Fibrosis after 16 weeks exposure was less pronounced in the lisinopril-treated groups, compared to the control groups in perivascular areas: group 1: 1 (0-2) vs. group 2: 2 (2-3), p<0.05 and group 3: 3 (2-3) vs group 4: 1 (1-2), p<0.05. After 20 weeks a significant difference was also shown in intersegmental fields: group 3: 2 (2-3) vs group 4: 1 (1-1.5), p<0.05. The amount of vessels was significantly less in both lisinopril-treated groups vs. their control groups: group 1: 15 (±10) vessels/field (v/f) vs group 2: 31 (±13), p<0.05 and group 3: 15 (±7) vs 42 (±8) v/f, p<0.05.

CONCLUSIONS

It can be concluded that the fibrotic and angiogenic effects of exposure of the peritoneum to bioincompatible dialysis solutions can be attenuated by administration of lisinopril in rats.
INTRODUCTION

Long-term peritoneal dialysis (PD) can lead to structural and functional changes of the peritoneum [1-4]. The morphologic changes consist of an increased thickness of the submesothelial compact collagenous zone of the parietal peritoneum, sometimes accompanied by loss of surface mesothelium [1,3]. Interstitial fibrosis can also be found in omental tissue [2]. Extensive vascular abnormalities have been described. These include not only subendothelial hyalinosis of arterioles, but also of the venules and small veins. [3,5]. Also an increased number of vessels has been found [2], especially in patients with ultrafiltration failure [4]. The thickness of the submesothelial compact zone was related to the duration of PD, the absence of mesothelium and the prevalence of vasculopathy [4]. A correlation has also been described between the number of peritoneal vessels and the fibrotic alterations [2]. These fibrotic and vascular abnormalities are also present in patients with encapsulating peritoneal sclerosis, but much more severe [2]. The increase in the number of peritoneal blood vessels causes an increase in the vascular peritoneal surface area, leading to ultrafiltration failure due to high glucose absorption rates, resulting in a rapid disappearance of the osmotic gradient [6].

Angiotensin-II is an important growth factor in the development of renal fibrosis [7]. Treatment with angiotensin converting enzyme (ACE) inhibitors and angiotensin-II (A-II) receptor blockers has shown marked renoprotective effects especially in patients with diabetic nephropathy [8-10]. The renal effects of angiotensin-II, namely the stimulation of extracellular matrix protein synthesis, are likely to be mediated by TGF-β. This has been shown for cultured rat mesangial cells [11], and in a model of obstructive nephropathy in mice [12]. Furthermore, treatment with ACE inhibitors attenuated the renal expression of TGF-β in patients with IgA nephropathy [13]. Acute studies with i.p. administered captopril in rats have reported increases in dialysate/plasma ratios of low molecular weight solutes and increased dialysate protein losses [14,15].

Intraperitoneal enalapril for 4 weeks reduced the thickness of parietal peritoneum in rats daily exposed to hypertonic glucose/lactate based dialysis solutions. This was associated with improved ultrafiltration and a lower D/P urea than in the dialysate only group [16]. Oral administration of quinapril for up to 56 days markedly reduced peritoneal thickening in a murine model of peritoneal sclerosis [17].

We hypothesized that ACE inhibition could attenuate the development of peritoneal fibrosis during chronic peritoneal exposure to dialysis fluid, similar to the effects of ACE inhibition in diabetic nephropathy on mesangial matrix expansion [18]. Therefore we investigated the effects of ACE inhibition on peritoneal fibrosis by oral administration of lisinopril in a chronic peritoneal exposure model in the rat.
MATERIALS AND METHODS

ANIMALS

Twenty-eight male Wistar rats (HSD, Harlan, Zeist, The Netherlands) were investigated in four groups, 7 rats per group. The rats were housed solitarily under controlled conditions (temperature 19°C, relative humidity 50±5%, 12/12 hour light/dark cycle) and fed standard chow (Hope Farms, Woerden, The Netherlands) and water ad libitum. All rats acclimatized for one week before insertion of a peritoneal catheter. The length of the catheter was adjusted for each rat and a titanium/silicone device (Rat-o-Port, MTINC, 7IS, Access Technologies, Norfolk Medical, Skokie, IL, USA) was attached to the catheter and implanted subcutaneously and adequate pain sedation was applied (buprenorfine 0.3 mg/mL). From the next day on, intraperitoneal infusion was achieved by daily puncture of the subcutaneous device implanted in the neck of the rat, using a 0.6 mm wing-end infusion set (microperfuscur, Vygon, France). 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 were infused daily with 60 ml/kg body weight 3.86% glucose containing dialysis solution via the Rat-o-Port, prior to infusion, the solution was warmed to 37°C. During the experimental period 3 rats were sacrificed before completion of the study because of blocked catheters. Peritoneal histology revealed signs of peritonitis (extensive infiltrates in all peritoneal biopsies) and thus the animals were left out of all analyses. Group 1 was also treated with lisinopril dissolved in the drinking water (pure substance, obtained from Astra Zeneca, 0.15 mg/mL) for 16 weeks (n=7), group 2 received only infusion for 16 weeks (control group, n=6), group 3 was treated with infusions and lisinopril for 20 weeks (n=7) and group 4 served as the control group (n=5) that received only infusions for 20 weeks after which the rats were killed by bleeding provoked by cardiac puncture. The study was performed in accordance with the regulations required by the local ethics committee for animal experimentation.

HISTOPATHOLOGICAL ASSESSMENT

Omental and parietal peritoneal tissue were obtained in each rat after sacrifice and these specimens were prepared 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/cosin and picro-sirius red F3B (PSR), providing a brick red staining of all fibrillary collagen. For visualization of vessels, adjacent sections were immunohistochemically stained for anti-α-smooth muscle actin (SMA-1, dilution 1:800, DAKO, Denmark), which is expressed in vascular smooth muscle cells and pericytes. A streptavidin-biotin-peroxidase method was used. The sections were deparaffinized in xylene and rehydrated in ethanol, followed by incubation with hydrogen peroxide 0.3% in methanol to block endogenous peroxidase activity. The onset of the staining sequence was a block with 10% normal goat serum followed by primary antibodies against αSMA (monoclonal, DAKO,
Glostrup, Denmark) for 60 min. Before this incubation with PBS-NHS containing 5% normal rat serum and horse radish peroxidase-conjugated streptABC complex (DAKO, Glostrup, Denmark) were applied to enhance the reaction and as conjugate. The peroxidase activity was detected with 1 mg/mL 3,3-diaminobenzidine tetrahydrochloride (Sigma, St Louis MO, USA) and 0.015% H$_2$O$_2$ in 50 mM Tris-HCl buffer, pH 7.6, yielding the brown coloration. All slides were counterstained with Mayer’s hematoxylin, dehydrated through a series of ethanol and mounted with Pertex mounting medium.

**Vessels and Fibrosis Scoring**

The number of vessels per field of peritoneal tissue section was counted in αSMA-stained omental tissue, using a light microscope (Leitz Dialux 20, Leica, Rijswijk, The Netherlands) with a 25x flat field objective (x10 ocular). Five non-overlapping fields from the upper left to the lower right were investigated throughout the specimen, in which all vessels were counted and measured.

Fibrosis was assessed by a semi-quantitative score and also quantitatively. The semiquantitative score was performed as previously described [19] using both omental and parietal sections stained with PSR. Scoring was as follows: 0=normal presence of fibrous tissue, 1=mild excess, 2=moderate, 3=severe excess, assessed in three areas of omental tissue: submesothelial (SM), perivascular (PV) and intersegmental (IS). These scorings were added to obtain an overall fibrosis score. The semi-quantitative scoring was assessed by two blinded observers and interobserver variability was less than 10%.

**Statistical Analysis**

Medians and interquartile ranges are given unless stated differently. Mann-Whitney $U$ tests were done to investigate differences between groups.

**Results**

Significant morphological differences were found between the experimental groups and their time-matched controls (Table 1).

In PSR stained tissue it was shown that fibrosis was less for intersegmental areas at both 16 and 20 weeks and in perivascular areas at 20 weeks. The overall fibrotic score was significantly less in the lisinopril treated animals compared to their controls.

Immunostaining with αSMA revealed a significantly smaller number of peritoneal vessels in lisinopril treated animals both at 16 and at 20 weeks compared to infusion only. Representative examples are shown in Figure 1.
Table 1: Quantification of numbers of vessels and amount of fibrosis in omentum of rats infused with buffered dialysate. Medians and ranges are given. This scoring of fibrosis was performed in submesothelial (SM), perivascular (PV) and intersegmental (IS) areas.

<table>
<thead>
<tr>
<th>Vessels/field</th>
<th>Group 1 Lisinopril 8 weeks</th>
<th>Group 2 controls 8 weeks</th>
<th>Group 3 Lisinopril 16 weeks</th>
<th>Group 4 controls 16 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosis (0-3)</td>
<td>15 (4-48)</td>
<td>31 (7-68)</td>
<td>15 (5-36)</td>
<td>42 (20-87)</td>
</tr>
<tr>
<td>SM</td>
<td>1 (0-2)</td>
<td>1 (1-2)</td>
<td>1 (1-2)</td>
<td>3 (2-3)</td>
</tr>
<tr>
<td>IS</td>
<td>1 (0-1)</td>
<td>2 (1-2)</td>
<td>1 (1-1.5)*</td>
<td>2 (2-3)</td>
</tr>
<tr>
<td>PV</td>
<td>1 (0-2)</td>
<td>2(2-3)*</td>
<td>1(1-2)*</td>
<td>3 (2-3)</td>
</tr>
<tr>
<td>Overall fibrotic score</td>
<td>1 (0-2)</td>
<td>2 (1-2)*</td>
<td>1 (1-2)*</td>
<td>3 (2-3)</td>
</tr>
</tbody>
</table>

*p<0.05 compared to time-matched controls

Discussion

The present study has shown that oral administration of lisinopril attracts fibrosis and the number of peritoneal blood vessels that can be induced by long-term administration of the conventional 3.86% glucose-based/lactate-buffered dialysis solution. These results are in accordance with those obtained after 4 weeks treatment with enalapril on the thickness of the submesothelial fibrous tissue layer [16].

In this study the protective effect on the development of fibrosis during peritoneal exposure to bioincompatible dialysis fluid was especially present in intersegmental areas at both 16 and 20 weeks and in perivascular areas at 20 weeks. In other studies in our chronic peritoneal exposure model in the rat we also found more effect of experimental strategies in the intersegmental and perivascular areas as the submesothelial areas did not show significant differences [unpublished results]. It is known that tissue renin-angiotensin system (tRAS) is important in the formation of fibrosis [20]. Angiotensin II promotes gene expression and synthesis of growth factors in mesangial cells and tubular epithelial cells [21]. These growth factors include platelet-derived growth factor [22], fibroblast growth factor, and transforming growth factor-β [23]. TGF-β stimulates the production of extracellular matrix, by inducing production of fibronectin, collagens, and proteoglycans [24]. Angiotensin II induces the expression of TGF-βmRNA and thus the inhibition of RAS might prevent tissue fibrosis.

Other publications on ACE inhibition in peritoneal exposure models have mainly focussed on the protective effect on fibrosis, especially the submesothelial thickness. In the present study also the amount of vessels was significantly less in tissues from rats treated with infusion and lisinopril compared to infusion only. At 16 weeks there was a reduction in the amount of vessels of 50% in the lisinopril treated animals compared to the control group and at 20 weeks even 65%. It has been proposed that the physiological actions of the RAS can be viewed as a coordinated effort to preserve tissues against acute ischemia [25]. When blood supply is restricted in a focal area, local activation of the tissue RAS induces not only the rapid
recruitment of the collateral circulation to limit the effect of decreased blood flow, but also contributes to cellular mechanisms for adaptation to the ischemic insult, allowing the cells to survive and recover.

Ultimately, if the ischemic injury is severe enough, angiotensin II promotes apoptosis of the most severely affected cells, thereby favoring the use of limited energy supplies to the still viable cells. As time proceeds, a slower process takes place to restore blood flow to ischemic areas through angiogenesis. The effect of angiotensin II in vivo was first demonstrated by incorporating it in a rabbit cornea where it produced a progressive formation of new vessels [26], later this was also demonstrated in mice [27]. These findings might explain the anti-angiogenic effect of the ACE inhibitor lisinopril that was found in the current study. Indeed it was demonstrated that ACE inhibition hampered vessel growth in the diabetic retina of mice, which suggests that RAS blockade prevents diabetes-induced neovascularization [28].

In PD patients Ripley et al. performed a study investigating the effect of oral and intraperitoneally administered enalapril on, amongst other things, functional characteristics [29]. The short-time nature of this study and the limited amount of patients could have accounted for the lack of any statistically significant effect on peritoneal function. Both a bigger group of patients and a longer-follow up would be necessary to investigate this effect. Also, such a study would likely have to be a cross-sectional analysis.

It can be concluded that the known profibrotic and angiogenic effects of exposure of the peritoneum to bioincompatible dialysis solutions in rats can be attenuated by administration of lisinopril. This might result in a better preservation of peritoneal function and might have implications for the clinical situation, but this will first have to be confirmed in PD patients.

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References


