Understanding and preventing the peritoneal damage caused by conventional dialysis solutions
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Chapter 9

Chronic Peritoneal Exposure to a Filter Sterilized Pyruvate Buffered Hypertonic Dialysis Solution with a Combination of Three Osmotic Agents

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Abstract

Background

Pseudohypoxia, a situation in which the intracellular NADH/NAD⁺ ratio is increased, is mediated partly via the polyol pathway. Peritoneal dialysis with glucose and lactate containing dialysates is likely to stimulate the polyol pathway, thus causing pseudohypoxia, which may stimulate the formation of different growth factors.

Methods

Different strategies have been used to reduce pseudohypoxia in peritoneal exposure to dialysis fluids in a chronic peritoneal exposure model in the rat: Firstly, by using pyruvate as buffer of PD fluid instead of lactate, pseudohypoxia might be reduced and this may protect against the development of peritoneal fibrosis and angiogenesis. Also, filter-sterilization instead of heat-sterilization can be used to investigate the influence of advanced glycation end products, and by using a combination of osmotic agents, the glucose exposure can be reduced. A filter-sterilized dialysis fluid with a combination of pyruvate as a buffer and aminoacid/glycerol/glucose 1% (PYRAGG, n=6) was daily i.p. infused in Wistar rats for a period of 20 weeks. Standard heat-sterilized lactate-buffered/glucose 3.86% fluid was infused in control rats (LH, n=6) and an extra group was included that was infused with this standard dialysate, but that had been filter-sterilized (LF, n=9). After 20 weeks, a standardized peritoneal analysis was performed and omental tissue was examined for presence of fibrosis (sirius red) and number of vessels per field (αSMA).

Results

Permeability characteristics were similar in both experimental groups. Histology revealed a significant difference in the amount of Sirius Red-positive tissue in submesothelial (PYRAGG: 0.5 (0.5-1) vs LF: 1.5 (0.5-2.5) vs LH: 2.25 (2-3), p<0.05 between all 3, and also in intersegmental areas (PYRAGG: 0.5 (0.5-1) vs LF: 1 (1-2) vs LF: 2.25 (1.5-3) p<0.05 between all 3. Also a significant difference was present between the groups in numbers of CD31-positive vessels per field (PYRAGG: 13±7 v/f vs LF: 23±7 v/f, LH: 32±9, p<0.01 between all 3).

Conclusions

In a previous study it was shown that pyruvate as a buffer in dialysis fluid protected against the formation of fibrosis and angiogenesis in a chronic peritoneal exposure model in the rat. In this study filter sterilization seemed probably as effective in prevention of these alterations. And finally a combination of filter-sterilized, pyruvate-buffered, aminoacids/glycerol/glucose 1% containing dialysate seemed to be even more effective in preventing the peritoneal damage caused by exposure to dialysis fluids.
INTRODUCTION

Long-term peritoneal dialysis can lead to structural and functional changes of the peritoneum [1,2]. The chronic exposure to unphysiologic dialysis fluids, especially glucose and/or glucose degradation products, are thought to play a pivotal role in their pathogenesis [3,4]. Glucose has direct toxic effects on the mesothelium [5], induces diabetiform neoangiogenesis [6,7] and leads to the formation of advanced glycosylation end products [8]. High glucose concentrations lead to activation of the polyol pathway for intracellular glucose degradation, which may cause an elevation of the NADH/NAD+ ratio, also called pseudohypoxia [9]. Hypoxia is a stimulus for angiogenesis. Lactate may contribute to the toxicity of glucose by influencing the NADH/NAD+ ratio [10]. Pyruvate can improve cellular function by decreasing the cytoplasmatic redox potential [11]. In a previous study [12], the use of pyruvate as a buffer in a 3.86% glucose dialysis solution showed a reduction in the number of peritoneal blood vessels of 50% compared to lactate buffered fluid.

Currently no single low molecular weight osmotic agent can replace glucose completely. Aminoacids can only been given once a day in a low concentration to avoid an excessive nitrogen load. The continuous use of glycerol in high concentrations may cause a hyperosmolar coma [13]. Therefore we investigated whether a pyruvate buffered dialysis solution, made hypertonic by combining low concentrations of aminoacids, glycerol and glucose (PYRAGG), and without glucose degradation products, would induce less peritoneal abnormalities than glucose/lactate based solutions, either heat- or filter sterilized. The study was done in the chronic peritoneal exposure model in the rat.

SUBJECTS AND METHODS

ANIMALS

Twenty-one male Wistar rats (Harlan, HSD, Zeist) with weights between 300–320 grams were randomly divided in three groups. All rats acclimatized one week before implantation of a peritoneal catheter (Intisil 7 french) attached to a titanium/silicone device implanted in the neck (Rat-o-Port, both obtained from UNO Roestvrijstaal BV Zevenaar, The Netherlands) under anesthesia (mix of ketamine, xylazine, atropine, 4:2:1, 1mg/kg BW). The first week the rats were infused with 1 mL/day of heparinized saline to allow for peritoneal healing and thereafter the experimental period started. One group (n=6) was infused with PYRAGG, another group (n=9) with lactate/glucose 3.86% containing filter sterilized dialysate (LF) and a control group (LH, n=6) with lactate/glucose 3.86% heat sterilized dialysis fluid (Dianeal, Castlebar, Ireland). The first two fluids fluids were prepared in the laboratory and the specific constituents are given in Table 1.

All animals were daily infused with 60 mL/kg BW for 20 weeks. After this period they underwent a standard peritoneal permeability analysis adapted for the rat [14] before sacrifice.
by cardiac puncture. Peritoneal tissue was obtained immediately thereafter. The study was performed in accordance with the regulations required by the local ethics committee for animal experimentation.

Table 1: Constituents of the three different dialysis solutions.

<table>
<thead>
<tr>
<th></th>
<th>PYRAGG</th>
<th>LF</th>
<th>LH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺ (mM)</td>
<td>132</td>
<td>132</td>
<td>132</td>
</tr>
<tr>
<td>Ca²⁺ (mM)</td>
<td>1.25</td>
<td>1.25</td>
<td>1.75</td>
</tr>
<tr>
<td>Mg²⁺ (mM)</td>
<td>0.5</td>
<td>0.4</td>
<td>0.75</td>
</tr>
<tr>
<td>Pyruvate (mM)</td>
<td>40</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>-</td>
<td>40</td>
<td>35</td>
</tr>
<tr>
<td>Chloride (mM)</td>
<td>95</td>
<td>95</td>
<td>102</td>
</tr>
<tr>
<td>Glucose (%)</td>
<td>1.10</td>
<td>4.20</td>
<td>3.86</td>
</tr>
<tr>
<td>Aminocids (%)</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycerol (%)</td>
<td>1.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pH</td>
<td>5.2-5.4</td>
<td>5.2-5.4</td>
<td>5.5</td>
</tr>
<tr>
<td>Osmolarity (mOsmol/L)</td>
<td>517</td>
<td>483</td>
<td>486</td>
</tr>
</tbody>
</table>

ASSAYS

Total dextran 70 was measured in all dialysate samples by high performance liquid chromatography [15].

The glucose concentration was assessed by the glucose oxidase-peroxidase assay (SMA II, Technicon, Terrytown, NJ, USA). Sodium was measured by an ion selective electrode (Hitachi H747, Boehringer Mannheim, Mannheim, Germany).

SPARa

The SPARa is based on the human standard peritoneal permeability analysis (SPA) [16], adapted for the rat [14]. In short, an intravenous infusion needle was inserted in the left lower lateral quadrant of the abdomen, whereafter a 4 hours dwell with 30 mL LH in all rats was performed. Dextran 70, 5 g/L (Hyskon, Medisan Pharmaceuticals AB, Uppsala, Sweden) was added to the test solution as a volume marker for the calculation of fluid kinetics.

Peritoneal solute and fluid kinetics were calculated. From the dilution of the volume marker the transcapillary ultrafiltration was calculated after the correction for incomplete recovery of fluid [17]. The MTACs of creatinine and urea were calculated according to Waniewski et al. [18]. Net ultrafiltration was defined as the change in intraperitoneal volume divided by the dwell time. Glucose absorption was estimated as the difference between the instilled and the recovered amount of glucose, in relation to the instilled quantity. Free water transport was estimated by the sieving of sodium expressed as dialysate-to-plasma (D/P) ratio of sodium. In all animals the lowest values were taken.
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) providing a brick red staining of all fibrillary collagen. For visualization of vessels, adjacent sections were immunohistochemically stained for α1 smooth muscle actin (SMA-1, dilution 1:800, DAKO, Denmark), which is expressed in vascular smooth muscle cells and pericytes. For detection of 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, Denmark). PBS-NHS containing 5% normal rat serum and horse radish peroxidase-conjugated streptABC complex (DAKO, 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% H2O2 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.

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.

The amount of fibrosis was measured by semiquantitative scoring of the extent of PSR staining of omental sections. Briefly, submesothelial (SM), perivascular (PV) and intersegmental (IS) areas of omental tissues were judged separately, using grades as 0=normal presence of fibrous tissue, 1=mild excess, 2=moderate, 3=severe excess of fibrosis, and also an overall fibrotic score was calculated. The semiquantitative scoring was assessed by two blinded observers and inter-observer variability was less than 10%.

Statistical Analysis

Results are expressed as medians and interquartile ranges. Analysis of variance was performed whereafter Mann-Whitney tests were performed to assess differences between groups.

Results

No differences between the groups with regard to peritoneal transport were present (Table 2). However, morphological analysis of peritoneal tissues showed significant differences (Table 3). More pronounced fibrosis was found in the LH group than in the two other groups. In the tissues
from the rats treated with chronic peritoneal exposure to PYRAGG, the fibrosis was significantly less than in the two other groups. The number of vessels was highest in the LH group, less in the LF group (28% reduction when compared to the lactate heat-sterilized group) and lowest in the PYRAGG infused animals (59% reduction when compared to the LH group).

Table 2: standard peritoneal permeability analysis adapted for the rat.

<table>
<thead>
<tr>
<th></th>
<th>PYRAGG (n=6)</th>
<th>LF (n=9)</th>
<th>LH (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCUFR (µL/min)</td>
<td>87.5 (64-104)</td>
<td>75 (51-119)</td>
<td>71 (28-132)</td>
</tr>
<tr>
<td>NUFR (µL/min)</td>
<td>51.5 (24-76)</td>
<td>50 (5-71)</td>
<td>50.5 (25-56)</td>
</tr>
<tr>
<td>Gluc abs (%)</td>
<td>58.5 (35-72)</td>
<td>63 (40-73)</td>
<td>52 (49-57)</td>
</tr>
<tr>
<td>D/P sodium (%)</td>
<td>88 (78-92)</td>
<td>88 (76-89)</td>
<td>87 (85-89)</td>
</tr>
</tbody>
</table>

TCUF: transcapillary ultrafiltration rate, NUFR: net ultrafiltration rate, Gluc abs: glucose absorption, D/P sodium: dialysate/plasma ratio of sodium

Table 3: Histological assessment of omentum: number of vessels and the amount of fibrosis are shown as assessed in different areas: PV: perivascular, IS: intersegmental, SM: submesothelial. Medians and interquartile ranges are given.

<table>
<thead>
<tr>
<th>Fibrosis</th>
<th>PYRAGG</th>
<th>LF</th>
<th>LH</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV</td>
<td>1 (0-1)</td>
<td>1.5 (0.5-1.5)</td>
<td>2.75 (2-3)</td>
</tr>
<tr>
<td>IS</td>
<td>0.5 (0.5-1)</td>
<td>1 (1-2)</td>
<td>2.25 (1.5-3)</td>
</tr>
<tr>
<td>SM</td>
<td>0.5 (0.5-1)</td>
<td>1.5 (0.5-2.5)</td>
<td>2.25 (2-3)</td>
</tr>
<tr>
<td>Overall Fibrotic Index</td>
<td>0.5 (0.5-1)</td>
<td>1.5 (1-2)</td>
<td>2 (3-3)</td>
</tr>
<tr>
<td>Number of Blood Vessels</td>
<td>11 (7-16)</td>
<td>21 (17-28)</td>
<td>31 (24-42)</td>
</tr>
</tbody>
</table>

*: p<0.05 PYRAGG compared to LF and LH, †p<0.03 LF compared to LH, ‡p<0.05 PYRAGG compared to LF

Discussion

An ideally biocompatible solution for peritoneal dialysis should not contain high concentrations of glucose [19-23], should be free of glucose degradation products [23,24], should not be buffered with high lactate concentrations [24], and must still be an effective solution with regard to fluid and solute removal. PYRAGG is such a solution. Its hypertonicity, created by a combination of low concentrations of glucose, glycerol and aminoacids allowed ultrafiltration rates similar to those observed using a 3.86% glucose solution and was well tolerated by the animals. It was filter-sterilized, so contained no GDPs. The solution was buffered with pyruvate, based on the results of in vitro studies [25,26] and those of a previous study by our group in the chronic peritoneal exposure model in the rat [12]. PYRAGG was compared with two control solutions, that is the 3.86% glucose/lactate/low GDP solution, made by filter sterilization the 3.86% glucose/lactate heat sterilized solution. Marked morphological differences between the groups were found with less vessels (28% reduction in the LF group and 59% reduction in the PYRAGG group when compared to LH) and less fibrosis in LF and PYRAGG than in LH.
These marked morphological differences were not accompanied by differences in peritoneal transport; the same was found in our previous study comparing pyruvate as a buffer with lactate [12]. Indeed, relationships have been described between the number of peritoneal vessels and peritoneal transport [27,28]. However, the vascular surface area is not only determined by the number of perfused microvessels, but also by their state of vasodilation or vasoconstriction. For example, animal experiments have suggested that the MTAC of low molecular weight solutes is not determined by splanchnic blood flow, but by splanchnic blood volume [29].

The present study allows insight in the possible contribution of various factors in dialysate contributing to peritoneal damage: hyperosmolality had no or only a minor effect in the development of fibrosis and angiogenesis. This is in accordance with the results of an old in vitro study on cultured mesothelial cells by Breborowicz et al. [30]. GDPs (comparison between LH & LF) had an effect on fibrosis, which has been reported to be influenced by transforming growth beta (TGFβ) and vascular endothelial growth factor (VEGF). Interestingly the effect of GDPs on the number of vessels was only minor. Glucose had a marked effect on the number of vessels per field and also a marked effect on fibrosis. The replacement of the lactate buffer by pyruvate was not specifically investigated in the present study, but in a previous one it led to a marked reduction of the number of blood vessels when 3.86% glucose was as an osmotic agent [12]. This study does not answer the question about the role of pyruvate compared to lactate in the absence of high glucose concentrations. In conclusion, (1) The present study has shown that solutions made hyperosmolar by a combination of osmotic agents is less ahrmfull for the peritoneum than glucose only, (2) GDPs are important especially with regard to peritoneal fibrosis, (3) The role of pyruvate vs lactate in the absence of high glucose concentrations needs to be investigated.

REFERENCES

Chapter 9


