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Effects of reducing the lactate and glucose content of PD solutions on the peritoneum. Is the future GLAD?

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Abstract

Background. Long-term peritoneal dialysis (PD) may lead to functional and morphologic changes in the peritoneal membrane, probably because of the continuous exposure to conventional dialysis solutions.

Methods. The morphologic changes include neoangiogenesis and fibrosis. The authors of this article developed a long-term peritoneal exposure model in rats, in which the morphological alterations could be induced after daily peritoneal infusion of a 3.86% glucose/lactate-buffered conventional PD solution.

Results and Conclusions. In the present article, a review of the model and of the results obtained with various available and experimental solutions is given. It appeared that high lactate concentrations contributed to the glucose-induced neoangiogenesis by pseudohypoxia. Glucose degradation products were probably more important in the induction of peritoneal fibrosis. The promising results of a combination of amino acids, glycerol and glucose, each in a low concentration, buffered with either pyruvate or bicarbonate/lactate, are presented and discussed. The combination of glycerol, amino acids and dextrose, dissolved in a bicarbonate/lactate buffer (GLAD), may be an option for a new generation of dialysis fluids.

Keywords: peritoneal dialysis solutions; glucose; glycerol; lactate; pyruvate

Introduction

Long-term peritoneal dialysis (PD) may lead to peritoneal membrane failure. Functionally, such failure is characterized by an insufficient ability to remove excess fluid from the body, which is ultrafiltration failure. Its prevalence is >30% in long-term PD patients [1,2]. An enlargement of the peritoneal vascular surface area, leading to a rapid disappearance of the osmotic agent, is almost always present under this condition [3]. Morphologically, the presence of

an increased thickness of the submesothelial zone as a result of the formation of fibrous tissue is the most prominent abnormality of the parietal peritoneum in long-term PD [4–6]. Also, the number of peritoneal microvessels increases with the duration of PD, and vascular abnormalities can be present [5–7]. These include diabetiform reduplications of the capillary basement membranes [8] and subendothelial hyalinosis, not only of arterioles but also of venules [6]. A relationship between the vascular peritoneal surface area and peritoneal solute transport rate has been found [9].

It is likely that the described peritoneal alterations are caused by the continuous exposure to bioincompatible PD solutions. The most important factors are probably the extremely high concentrations of glucose and the presence of glucose degradation products (GDP) in the conventional dialysis solutions [10]. This has caused interest in strategies to improve their biocompatibility. Most studies have been done *in vitro*, but the translation of their results to clinical PD is often difficult and sometimes impossible. Therefore, this study aimed to develop a PD model in rats that would resemble the situation in PD patients as much as possible. To achieve long-term exposure, the model lasted for 20 weeks. This is much longer than most models at that time, which mainly used a period of 4 weeks.

Description of the model

The model has been developed using male Wistar rats with an average body weight (BW) of 280–300 g. They were specified pathogen free and housed solitarily under controlled conditions: temperature 19°C, relative humidity $50 \pm 5\%$ and 12/12 light/dark cycle. Their food consisted of standard chow and water *ad libitum*. All rats acclimatized for 1 week before insertion of a peritoneal catheter. The operation was done under anaesthesia by intramuscular administration of a mixture of ketamine, xylazine and atropine. A Rat-o-Port (a device for repeated punctures) was implanted subcutaneously in the neck and the attached silicone catheter (lumen 1.1 mm, length was adjusted per rat) with one Dacron cuff was tunnelled subcutaneously over the left flank, after which it was inserted into the peritoneal cavity proximal of the umbilicus. Adequate pain sedation

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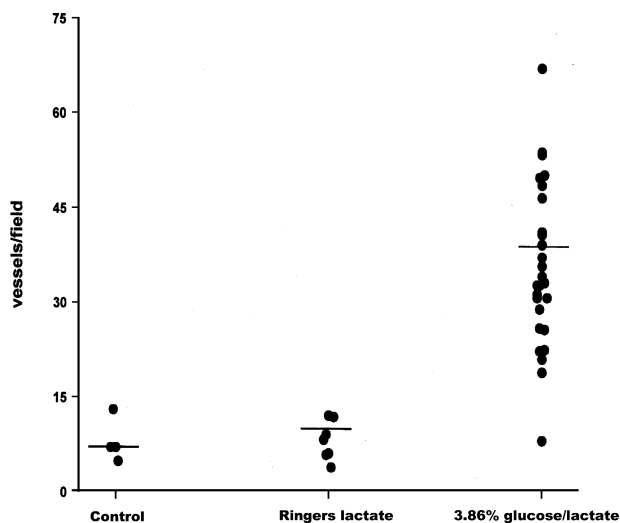
Fig. 1. The number of peritoneal blood vessels per high power field after 20-week exposure to Ringers lactate and to a 3.86% glucose/lactate-buffered PD solution (Dianeal®).

was applied using buprenorfine 0.3 mg/mL. Recovery occurred during 1 week following the catheter implantation with daily infusion of 1 mL heparinized saline (5 IU/mL in 0.9% NaCl) through the Rat-o-Port. The intervention period started directly after recovery. The interventions consisted of daily infusion of heparinized dialysis solutions, 60 mL/kg BW per day, preheated to $37°C$. The animals were awake during this procedure. No drainage was applied, to prevent blockage of the catheter. Instead, the infused solution was allowed to be absorbed overnight.

The model is different from clinical PD in the following ways: (1) it is an exposure model, (2) the rats are not uraemic, (3) animals with peritonitis were taken out of the study and (4) the maximum duration of exposure was 20 weeks, instead of >4 years. However, the lifetime of a rat is ~2.5 years. Twelve to twenty weeks of exposure therefore represents 9–15% of its lifetime. Extrapolation to the human situation equals a period of 7–11 years. The authors therefore think that the results obtained with this model are likely to represent the situation in humans. The model makes it possible to perform functional and morphological studies. In this article, only the results of the morphological studies have been presented.

Exposure to a conventional 3.86% glucose solution

Three groups of rats were studied. The experimental group received daily infusions of Dianeal® (3.86% glucose), one of the control groups received Ringers lactate and the other control group had no catheter or infusion [11]. The results on the number of blood vessels are shown in Figure 1. It was evident that the group exposed to Dianeal® had the most severe fibrosis and the largest number of peritoneal vessels. Electron microscopy of the vessels showed extensive lamination of their basement membrane [12], similar to general findings in diabetic microangiopathy and specifically in peritoneal microvessels of long-term PD patients [8]. The parietal peritoneum also showed a marked subme-

sothelial collagenous rind. Exposure to Ringers lactate did not induce peritoneal alterations (Figure 2, Tables 1 and 2).

The above findings are the reason that Dianeal® 3.86% glucose has always been included in the later studies on new PD solutions.

Exposure to a filter-sterilized 3.86% glucose, lactate-buffered solution

The only difference between this solution and the conventional PD solutions is the absence of GDPs because of the filter sterilization applied. A comparison was made between rats exposed for 20 weeks to the filter-sterilized solution, containing 3.86% glucose, and those exposed to the conventional PD solution (Dianeal® 3.86% glucose). The number of blood vessels was significantly lower in the animals exposed to the filter-sterilized solution than in the conventional solution ($P < 0.05$). However, the reduction was modest as shown in Table 1. Also, fibrosis was less in the animals exposed to filter-sterilized solution than in the conventional group ($P < 0.05$), as shown in Table 2.

Exposure to a 3.86% glucose solution buffered with bicarbonate and lactate

A commercially available dialysis solution buffered with bicarbonate 25 mmol/L and lactate 15 mmol/L (G-BL), Physioneal®, was compared with Dianeal® (G-L). In both, the glucose concentration was 3.86%, but G-BL has a pH of 7.4 and a lower content of GDPs. The average number of vessels after exposure to G-BL was lower than that in the animals exposed to G-L (Table 1). Also the amount of fibrosis was less on G-BL than on G-L (Table 2). The morphologic comparison is given in Figure 3. A reduction in the number of blood vessels on G-BL was also found in two other studies after 9- to 10-week [13] and 12-week exposure [14]. In the latter study also, a decreased staining for vascular endothelial growth factor (VEGF) and a reduction of mesothelial damage were present.

The pseudohypoxia hypothesis

The most important differences between Dianeal® and Physioneal® are the lower lactate concentration and the lower content of GDPs in the latter. The role of GDPs in peritoneal neoangiogenesis is probably limited, because of their low concentrations compared to those of glucose, which are 5000–10 000 times higher. Also the results of the studies discussed so far suggest that GDPs are probably more important for fibrosis than for neoangiogenesis. *In vitro* studies comparing lactate with pyruvate suggest an additional effect of lactate on glucose toxicity. Pyruvate induces less cytotoxicity to peritoneal macrophages and cultured mesothelial cells than lactate [15]. This could not only be attributed to the lower pKa of pyruvate making it a weaker buffer but also to its ability to scavenge oxygen radicals [16]. Pyruvate also causes less stimulation of the intracellular degradation of glucose in the polyol pathway [17]. These data suggest that lactate may contribute to the toxicity of glucose. We hypothesized
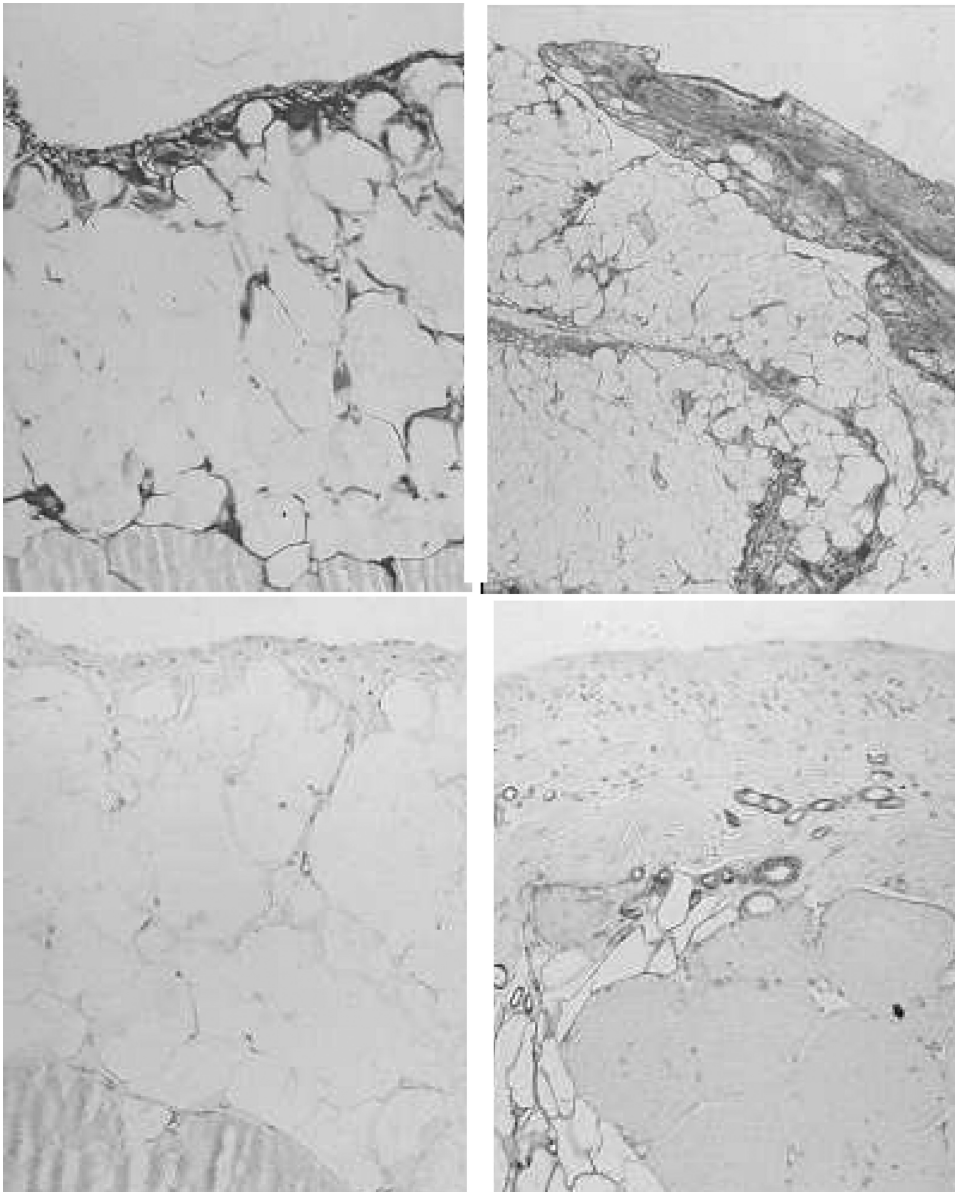
Fig. 2. Parietal peritoneum after 20-week exposure to Ringers lactate (left panels) and a 3.86% glucose/lactate dialysis solution (right panels). The upper panels are stained with picosirius red, which indicates the amount of fibrillary collagen. The lower panels are stained with collagen IV, which stains vascular basement membranes.

the presence of a mechanism leading to an effect on the intracellular $NADH/NAD^+$ ratio. This ratio increases under conditions of hypoxia and is a manifestation of cellular ischaemia. During the cellular degradation of glucose, NADH is formed from NAD^+ both in the glycolysis and in the polyol pathway. The resulting increase in the $NADH/NAD^+$ ratio is reversed by the conversion of pyruvate into lactate by lactate dehydrogenase. NAD^+ is also regenerated in the citric acid cycle. The authors hypothesize that the presence of extremely high lactate concentrations, as present in conventional dialysis solutions, leads to an increase in intracellular lactate, which inhibits the NAD^+ regeneration, thereby leading to a further increase

in the intracellular $NADH/NAD^+$ ratio (Figure 4). This mimics hypoxia. An increase in the $NADH/NAD^+$ ratio in hyperglycaemic patients with diabetes mellitus has therefore been called pseudohypoxia [18]. Hypoxia is the most important stimulus for the secretion of VEGF [19]. VEGF is an important growth factor for angiogenesis in patients with diabetes mellitus [20], especially in diabetic retinopathy [21,22]. Previously, it was found that VEGF in peritoneal effluent is locally produced, that it correlated with small solute transport [23] and increased with the duration of PD [24].

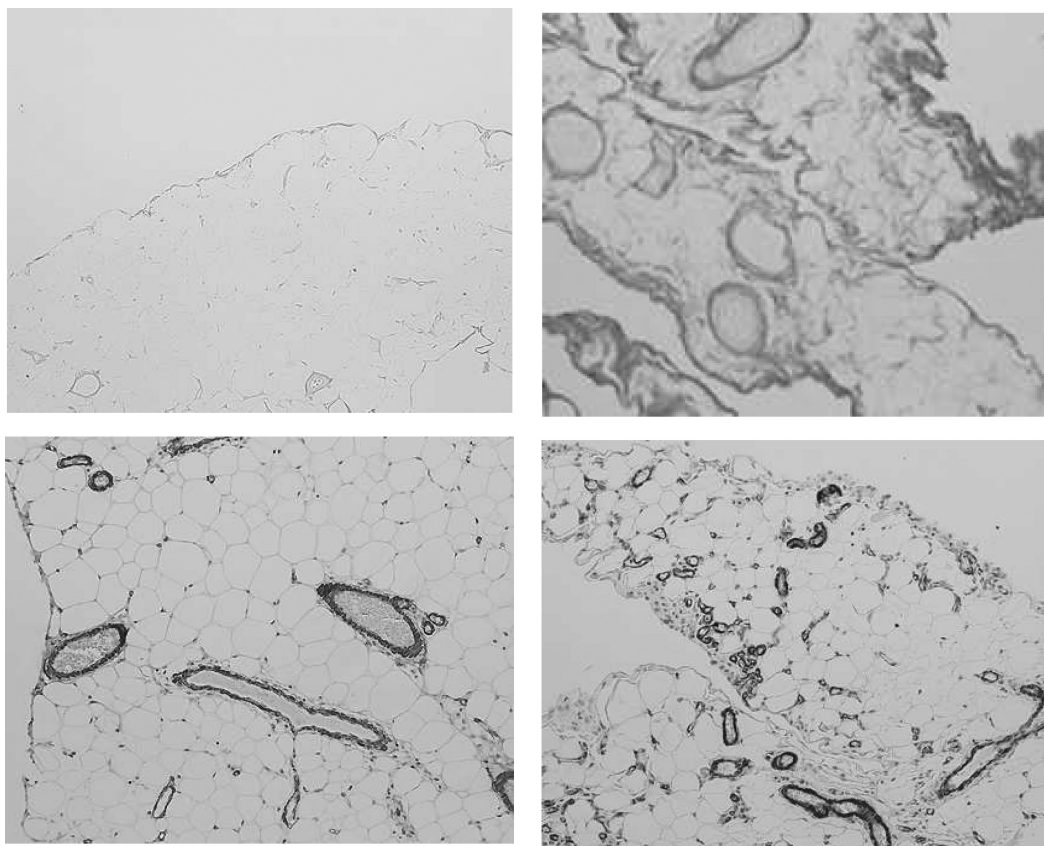This hypothesis prompted the authors to explore the effects of replacing the lactate buffer by pyruvate [25].

Fig. 3. Omentum after 20-week exposure to a 3.86% glucose/bicarbonate/lactate PD solution (Physioneal®, left panels) and a 3.86% glucose/lactate dialysis solution (Dianeal®, right panels). The upper panels are stained with picosirius red, which indicates the amount of fibrillary collagen. The lower panels are stained with alpha smooth muscle actin, which were used for vessel counting.

Table 1. Number of omental vessels per high-power microscopic field in the various studies and the percentage reduction induced by the various experimental solutions compared to a combined 3.86% glucose, lactate-buffered dialysis solution (Dianeal®)

	Experimental solutions	3.86% Glucose-based lactate-buffered solution	% Reduction compared to 3.86% G, L, GDP↑
Ringers lactate	8	35	77
3.86% G, L, GDP = 0	21	31	32
3.86% G, B/L, GDP↓	25	37	32
3.86% G, Pyragg, GDP↑	16	37	57
Pyragg, GDP = 0	11	31	64

GDP, glucose degradation products.

Table 2. Semiquantitative assessment of omental submesothelial fibrosis in the various studies

	Experimental solutions	3.86% Glucose-based lactate-buffered solution
Ringers lactate	0.7	1.5
3.86% G, L, GDP = 0	1.5	2.3
3.86% G, B/L, GDP↓	0.9	1.5
3.86% G, Pyragg, GDP↑	1.0	1.0
Pyragg, GDP = 0	0.5	2.3

GDP, glucose degradation products.
A comparison is made with a conventional 3.86% glucose/lactate-buffered dialysis solution (Dianeal®).
The score can range from 0 (no excess fibrosis) to 3 (most severe fibrosis).

Exposure to a heat-sterilized 3.86% glucose and pyruvate-buffered dialysis solution

According to the pseudohypoxia hypothesis of the authors, a comparison was made between 20-week daily exposure to 3.86% glucose Dianeal® and a heat-sterilized similar solution, in which only lactate was replaced by 35 mmol/L pyruvate. The latter solution was prepared in the hospital pharmacy and likely contained a similar amount of GDPs compared with Dianeal®. The hypothesis made that the elevated intracellular NADH/NAD$^+$ ratio is reduced using pyruvate was supported by the finding that the plasma betahydroxybutyrate/acetoacetate ratio was lower in the pyruvate group (3.1 ± 1.8) compared to the lactate-exposed animals (6.8 ± 6.4, $P = 0.07$). Exposure to pyruvate was associated with a reduction in the number of vessels ($P < 0.001$). This is shown in Table 1. Furthermore, the pyruvate-exposed animals had a larger total surface area of the vessels, a larger luminal area and a lower wall/total ratio than the lactate-exposed group. This suggests that pyruvate leads to some vasodilation. The differences in the amount
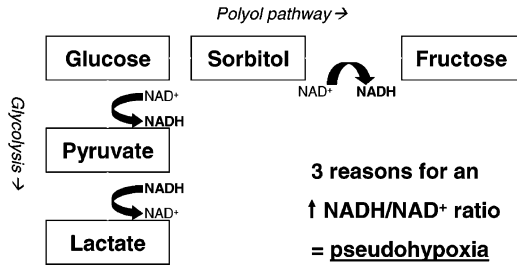
Intracellular high glucose

Fig. 4. A schematic representation of the intracellular glucose breakdown and its consequences for the NADH/NAD$^+$ ratio. See the text for further explanation.
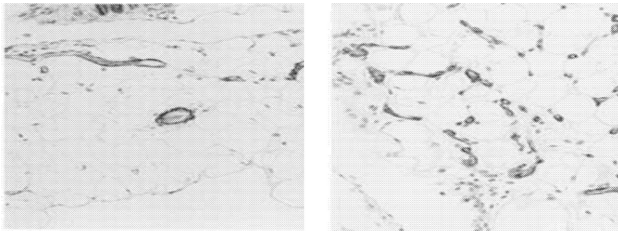

Fig. 5. Omentum after 20-week exposure to a 3.86% glucose/pyruvate-buffered solution (left panel) and a 3.86% glucose/lactate dialysis solution (Dianeal®, right panel). Both panels are stained with alpha smooth muscle actin, which was used for vessel counting.

of fibrosis were marginal (Table 2). A representative picture is shown in Figure 5. These data suggest that other factors, for example, GDPs, may be more relevant for the fibrotic alterations. A role for GDPs in peritoneal fibrosis was also likely in another study in rats comparing the contributions of different factors in dialysis solutions to the morphologic alterations after 10-week exposure [26].

Exposure to a filter-sterilized, pyruvate-buffered hypertonic dialysis solution with a combination of three osmotic agents

The results obtained with the pyruvate buffer described in the previous section, i.e. a reduced number of vessels, but with little effect on peritoneal fibrosis, led to the development of a theoretically ideal dialysis solution. This pyruvate-buffered solution contained three osmotic agents, each in a low concentration, and was filter sterilized. The osmotic agents were amino acids 0.5%, glucose 1.1% and glycerol 1.4%, leading to an osmolarity of 517 mosmol/L. This is even higher than that of 3.86% Dianeal®, which is 486 mosmol/L. The pH of the solution ranged from 5.2 to 5.4. This so-called Pyragg solution was used for daily exposure during 20 weeks, whereas the control group received exposure to 3.86% Dianeal®.

The exposure to Pyragg was well tolerated. A representative view of the morphology is given in Figure 6. Pyragg caused a similar reduction in blood vessels to the
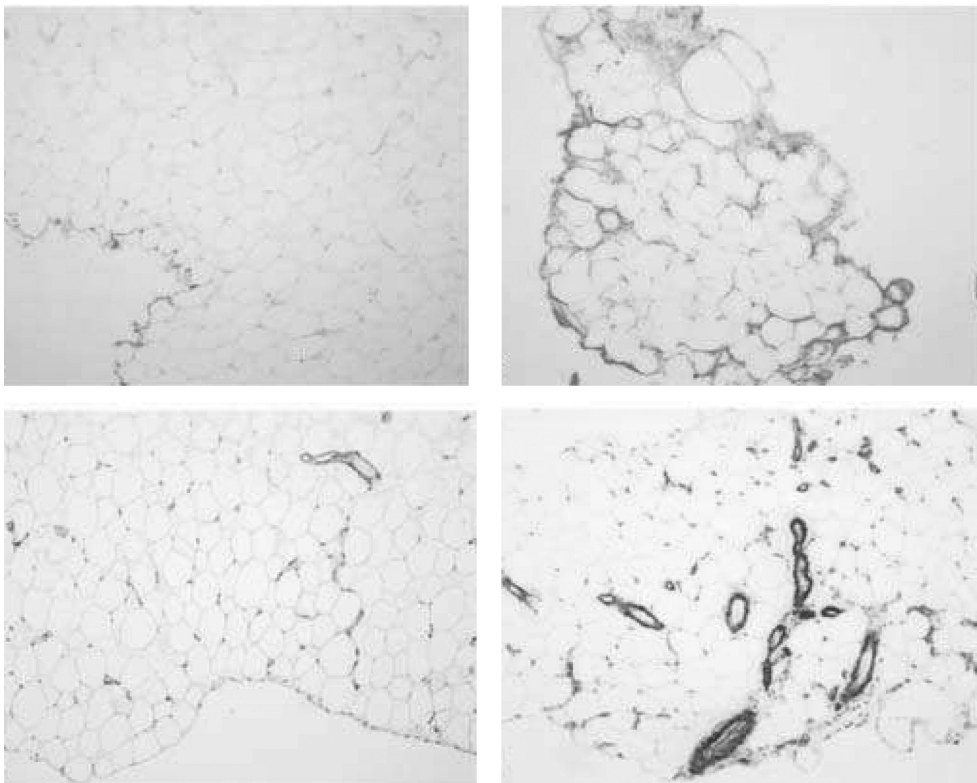

Fig. 6. Omentum after 20-week exposure to a pyruvate-buffered solution with a combination of three osmotic agents (Pyragg, left panels) and a 3.86% glucose/lactate dialysis solution (Dianeal®, right panels). The upper panels are stained with picosirius red, which indicates the amount of fibrillary collagen. The lower panels are stained with alpha smooth muscle actin, which were used for vessel counting.

Table 3. Comparison of the different solutions

Solution	Osmotic agents	Buffer	GDPs	pH	Osmolarity
GLAD	1.4% glycerol, 0.5% amino acids, 1% dextrose	Bicarbonate/lactate	Low	7.4	512
Physioneal®	3.86% dextrose	Bicarbonate/lactate	Low	7.4	486
Dianeal®	3.86% dextrose	Lactate	High	5.2	486
Buffer	None	Bicarbonate/lactate	None	7.4	266

GDP, glucose degradation products.

pyruvate-buffered solution (Table 1) but a greater reduction in fibrosis (Table 2). These results clearly show that hypertonicity *per se* is unlikely to be involved in the development of fibrosis and neoangiogenesis. They also suggest that a 60% reduction in the number of peritoneal blood vessels compared to conventional dialysis solutions, is possible using these experimental biocompatible PD solutions. In addition, a low concentration of GDPs had a positive effect on the severity of peritoneal fibrosis.

The glycerol, amino acids, dextrose study

Despite the theoretical advantages of using pyruvate as a buffer and the beneficial results described earlier, it is very unlikely that it will be used in clinical practice. The main reason for this is the existence of a patent. Therefore, the possibility of the addition of glycerol, amino acids and dextrose in low concentrations to the bicarbonate/lactate buffer of Physioneal® (GLAD) was investigated. The GLAD solution was prepared by Baxter R&D (Nivelles, Belgium) in a double bag system, similar to Physoneal®, and heat sterilized. A comparison between the different solutions is given in Table 3. GLAD was investigated and compared to the other solutions in rats with chronic renal failure.

The number of animals exposed was still small, but GLAD tended to induce only limited peritoneal fibrosis, similar to Physioneal® and the buffer, and less than Dianeal®. In addition, the number of peritoneal vessels tended to be smaller: GLAD ($n = 15$), buffer ($n = 16$), Physioneal® ($n = 22$) and Dianeal® ($n = 25$). Because of the limited number of the rats, these findings must be interpreted as preliminary, but to date they confirm the results of previous studies by the authors in rats with normal renal function.

Conclusion

These studies in the long-term peritoneal exposure model in the rat have shown that—similar to the situation in long-term PD patients—peritoneal neoangiogenesis and fibrosis can be induced by exposure to conventional PD solutions. The lactate buffer contributes to the toxicity of glucose with regard to glucose-induced neoangiogenesis. This effect is probably mediated by intracellular pseudohypoxia leading to production of VEGF. It explains why lowering the lactate concentration or replacing it by pyruvate has beneficial effects. The results also suggest that GDPs may be important for the induction of peritoneal fibrosis.

Hyperosmolarity had no effects on angiogenesis and fibrosis. The use of a combination of three osmotic agents, all in low concentrations, has given promising results. It should be realized that the GLAD solution, as investigated by the authors of this study, was compared to 3.86% glucose solutions. For use in patients, GLAD solutions similar to 2.27% and 1.36% glucose should also be prepared. It can therefore be concluded that when the data obtained with GLAD can be confirmed, it may be an ideal candidate for a new generation of dialysis fluids aimed at minimizing peritoneal damage, also after long-term exposure to PD.

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