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Brief Report

Peritoneal dialysis fluid-induced changes of the peritoneal membrane are reversible after peritoneal rest in rats

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Abstract

Background. Peritoneal dialysis (PD) is associated with functional and structural alterations of the peritoneal membrane. However, the (ir)reversibility of these pathological changes of the peritoneum is not understood fully.

Methods. In an experimental PD model, rats ($n = 15$) received daily 10 ml conventional glucose containing PD fluid, via peritoneal catheters connected to implanted subcutaneous mini vascular access ports. After 5 weeks of treatment, the first group of animals (PDF; $n = 10$) was sacrificed, while peritoneal catheters of the remaining group of rats (PD-rest; $n = 5$) were removed 1 week later. The latter group (PD-rest) was sacrificed 12 weeks after removing catheters. At both time points, untreated rats were included as controls. Cellular and morphological parameters were analysed by light and electron microscopy.

Results. Rats exposed to PD fluid for 5 weeks showed a severe angiogenesis in various peritoneal tissues. Peritoneal rest resulted in a significant reduction in blood vessel density in visceral (mesentery, $P < 0.05$), but not in parietal peritoneum. Five weeks' exposure to PD fluid resulted in a profound fibrosis in the parietal peritoneum, whereas the degree of fibrosis was significantly reduced in the PD-rest group ($P < 0.02$). Daily exposure to PD fluid induced a higher number of mast cells in the omentum compared with untreated rats, whereas peritoneal rest normalized the increased mast cell density completely ($P < 0.03$). Likewise, continued PD fluid instillation evoked a strong omental milky spot response, which was returned to the control level after peritoneal rest ($P < 0.009$). Furthermore, the number of mesothelial cells on the liver was significantly increased in rats treated with PD fluid, whereas animals from the PD-rest group had a lower number of mesothelial cells, although this was not statistically significant ($P = 0.08$). Finally, as evidenced by electron microscopy, daily exposure to PD fluid resulted in severe damage to the mesothelial cell layer covering the peritoneum, whereas this cell layer was completely recovered after peritoneal rest.

Conclusions. We show that PD fluid-induced cellular and morphological alterations of the peritoneal membrane are generally reversible.

Keywords: cellular alterations; morphological alterations; peritoneal dialysis fluids; peritoneal membrane; peritoneal rest

Introduction

Conventional peritoneal dialysis fluids (PDF) induce severe pathological alterations of the peritoneal membrane during long-term treatment. In addition to glucose and glucose degradation products, acidity in combination with lactate buffer and the accumulation of advanced glycation end-products are thought to be responsible for the bioincompatibility of these conventional PDF [1–5].

In an established rat model for peritoneal dialysis, we have shown that daily PDF instillation for 5 weeks led to cellular alterations of the peritoneal membrane, such as increased number of omental mast cells and milky spots as well as damage to the mesothelial cell layer covering the peritoneum [6,7]. In addition, severe morphological changes, including angiogenesis and fibrosis, occur in various peritoneal tissues. However, the (ir)reversibility of these peritoneal changes after terminating the treatment (peritoneal rest) is barely documented [8,9].

To evaluate the effects of peritoneal rest on the cellular and morphological/structural changes of the

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peritoneal membrane, rats were exposed to standard PDF for 5 weeks. After the treatment, rats were divided in two groups: the first group was immediately sacrificed, while the second group was subjected to a peritoneal rest for a period of 3 months. Using light and electron microscopic approaches, we performed a quantitative morphometric analysis of the peritoneal membrane, with emphasis on angiogenesis, fibrosis, omental activation and the condition of the mesothelium.

Subjects and methods

Animals

Throughout the study we used male Wistar rats (Harlan CPB, Horst, the Netherlands), weighing 280–300 g at the start of the experiment. The rats were acclimatized for 1 week before any treatment. Animals were maintained under conventional laboratory conditions and were given free access to water and food. The experiment was reviewed and approved by the local ethical committee on the use of laboratory animals.

Experimental design

Experimental animals ($n = 15$) received glucose containing PDF (Dianeal® PD4; 3.86% glucose, pH 5.5; Baxter R&D, Utrecht, the Netherlands) for 5 weeks. Thereafter, rats were divided randomly in two groups: the first group (PDF; $n = 10$) was sacrificed immediately after 5 weeks' PDF treatment, while PDF instillation was stopped in the remaining group of rats (PD-rest; $n = 5$). Peritoneal catheters were removed 1 week later. The latter group (PD-rest) was sacrificed 12 weeks after removing catheters, i.e. after 3 months of peritoneal rest. We included two control groups (without catheter and PDF exposure) for each time point. The first control untreated animals (C1; $n = 7$) were sacrificed at the same time as rats from the PDF group, while the second control untreated group was killed 3 months later (C2; $n = 5$).

Experimental peritoneal dialysis model

Peritoneal catheters connected to implanted subcutaneous mini vascular access ports were operatively placed in all experimental animals (except controls), as described previously [6,7]. During the first week post-operation, all experimental animals, except controls, received 2 ml saline with 1 U/ml heparin to allow wound healing. PDF were given daily, for 5 weeks, between 09.00 and 12.00 without any addition of heparin or antibiotics.

Morphological analysis

Omentum and mesentery. The number of blood vessels was quantified in stretched preparations of both the omentum (two sections of ~4 cm^2/rat) and in the mesentery (the most distally situated loop entirely) after staining with toluidine blue, as described previously [6,7]. Omental tissues were inspected for the presence of bacteria and/or adherent neutrophils in order to exclude silent peritonitis. In addition, since omental milky spots (local aggregates of immune cells) are the major route through which leukocytes migrate into the peritoneal cavity and because their size and number reflect the activated state of the peritoneum [10], we determined their number and size by light microscopy, as described previously [6,7]. Total milky spot surface area was calculated by multiplying both parameters. In 10 randomly selected areas of the omentum and mesentery, mast cells were counted and expressed as a number of cells per square millimetre.

Parietal peritoneum. The thickness of the submesothelial extracellular matrix (ECM) was determined after van Gieson staining (Merck KGaA, Darmstadt, Germany) and the average of 10 independent measurements was calculated for each section and expressed in microns [6,7]. The number of submesothelial blood vessels was quantified using anti-CD31 (PECAM; Serotec, Oxford, UK) and expressed as the number of vessels per millimetre length of the mesothelial cell layer [7].

Liver. Mesothelial liver imprints were dried and stained by May-Grünwald/Giemsa, as described before [6,7]. The number of cells per 0.1 mm^2 area was counted and the average number of cells in 16 areas was calculated for each slide and expressed as cells per square millimetre.

Electron microscopy of peritoneal tissues

Portions of the dissected omentum, mesentery, diaphragm, liver and parietal peritoneum from at least three animals per study group were prepared for electron microscopy according to standard procedures [6].

Statistical analysis

All data shown are expressed as medians and 25th to 75th interquartile ranges. Data were analysed statistically using either the non-parametric Kruskal–Wallis or Mann–Whitney U tests. A P-value of <0.05 was regarded as significant.

Results

Angiogenesis

Daily exposure to PDF induced severe angiogenesis in the omentum, mesentery and parietal peritoneum compared with both control groups (Table 1). Peritoneal rest significantly reduced the number of blood vessels in the mesentery ($P < 0.05$), although still more blood vessels were found in the PD-rest group compared with C1 ($P < 0.005$) or C2 groups ($P < 0.02$). In contrast, peritoneal rest had no apparent effect on the PDF-induced angiogenesis in the omentum ($P = 0.14$) or in the parietal peritoneum ($P = 0.62$).

Fibrosis

The thickness of the submesothelial ECM of the parietal peritoneum was increased markedly after the instillation of PDF compared with both control groups ($P < 0.002$; Table 1). Peritoneal rest for 3 months significantly reduced the degree of fibrosis in the parietal peritoneum ($P < 0.02$). However, the submesothelial ECM was still thickened upon PDF treatment followed by peritoneal rest compared with C1 ($P < 0.05$) or C2 groups ($P < 0.008$).

Table 1. Morphometric parameters of peritoneal tissues

Parameter	PDF ($n=10$)	PD-rest ($n=5$)	C1 ($n=10$)	C2 ($n=5$)
Angiogenesis				
Omentum (vessels/cm^2)	294 (263–334)[a]	118 (107–297)[b]	18 (15–54)	17 (13–25)
Mesentery (vessels/cm^2)	584 (514–608)[a,c]	453 (403–501)[b]	38 (32–49)	35 (34–38)
Parietal peritoneum (vessels/mm)	2.8 (2.8–3.1)[a]	2.5 (2.2–2.9)[b]	0.2 (0–0.2)	0.2 (0–0.4)
Thickness of ECM				
Parietal peritoneum (μm)	27.0 (26.9–27.5)[a,c]	17.6 (16.0–24.8)[b]	13.4 (13.0–15.0)	13.0 (13.0–13.5)
Cellular changes				
Omental milky spots/cm^2	30 (26–36)[a]	18 (17–31)[b]	8 (4–14)	5 (5–5)
Area/milky spots (mm^2)	0.50 (0.40–0.58)[a,c]	0.25 (0.23–0.29)	0.17 (0.09–0.20)	0.10 (0.03–0.28)
% Omental milky spots surface	17.4 (13.4–21.6)[a,c]	4.5 (2.7–8.5)[b]	1.2 (0.4–2.6)	0.8 (0.2–1.4)
Omental mast cells/mm^2	53 (29–99)[a,c]	21 (19–24)	18 (12–26)	20 (12–30)
Mesenteric mast cells/mm^2	89 (70–100)	70 (63–74)	63 (54–91)	62 (60–70)
Mesothelial regeneration				
Cell density on liver/mm^2	2780 (1270–1430)[a]	1020 (980–1150)[b]	880 (788–928)	830 (820–910)

[a]PDF *vs* C1 and C2: $P<0.02$.
[b]PD-rest *vs* C1 and C2: $P<0.05$.
[c]PDF vs PD-rest: $P<0.05$.

Cellular alterations

Chronic instillation of PDF induced a strong milky spot response in the omentum, as both the number and size of milky spots (and subsequently the total milky spot area) were significantly increased compared with both control groups (Table 1). After 3 months' peritoneal rest, PDF-induced milky spots were substantially smaller ($P<0.02$), but still present, resulting in a significant reduction in the total milky spot surface area compared with rats exposed to PDF ($P<0.009$). Peritoneal rest, however, did not completely normalize the PDF-induced milky spot response in the omentum (PD-rest *vs* C1: $P<0.03$; PD-rest *vs* C2: $P<0.02$).

Exposure to PDF resulted in an increased number of mast cells in the omentum compared with both control groups (Table 1). Peritoneal rest for 3 months completely normalized the PDF-induced mast cell response ($P<0.03$). In contrast to the omentum, no differences were found in the number of mast cells in the mesenteric tissues among the four groups ($P=0.64$).

Damage to the mesothelium

The number of mesothelial cells on the liver was increased significantly in rats treated with PDF compared with C1 ($P<0.0001$) or C2 groups ($P<0.003$; Table 1). Peritoneal rest for 3 months resulted in a reduction in the PDF-induced mesothelial regeneration response; however, this was not statistically significant, due to one outlying observation ($P=0.08$). Subsequently, a higher number of mesothelial cells were found on the liver of rats from the PD-rest group compared with C1 ($P<0.03$) and C2 animals ($P<0.02$).

The mesothelial cell layer on the peritoneal surface of both visceral and parietal peritoneum of control animals showed a normal (intact) appearance; thus, mesothelial cells and their microvilli were present (Figure 1A). In contrast to control animals, chronic exposure to PDF resulted in severe damage to the mesothelial cell layer covering the peritoneal

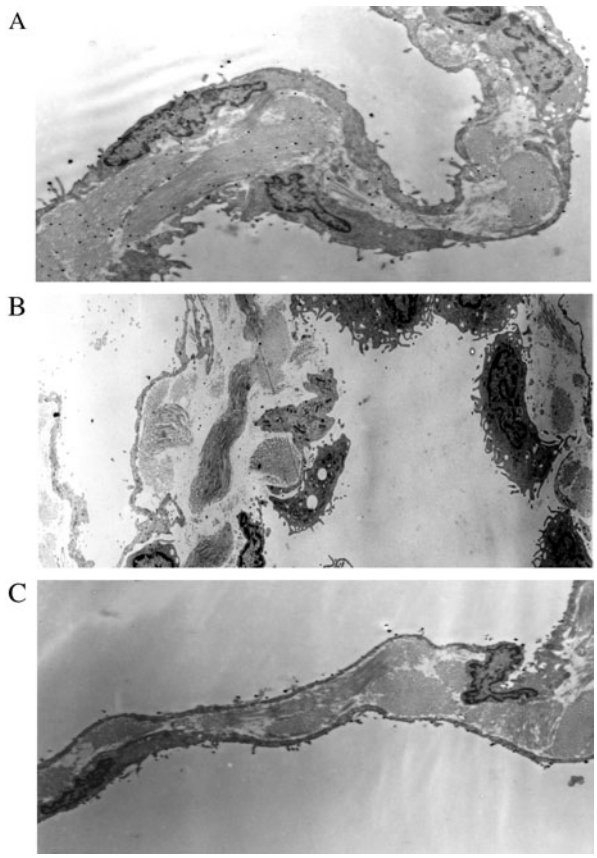

Fig. 1. Representative electron micrographs of the mesothelial cell layers covering the omentum of control rats (**A**), rats exposed to the conventional glucose containing PDF (**B**) and animals treated with PDF followed by a peritoneal rest (**C**). MC, mesothelial cell; MØ, macrophage.

membrane, which was characterized by loss of microvilli, vacuolization or complete loss of mesothelial cells as well as the adhesion of macrophages (Figure 1B). Exposure to PDF followed by 3 months' peritoneal rest recovered the mesothelium completely (Figure 1C).

Discussion

The peritoneal membrane responds progressively to PDF by inducing angiogenesis and fibrosis as well as by increasing the number of omental mast cells, omental milky spots and mesothelial cells on the liver and by damaging the mesothelial cell layer, as evidenced by light and electron microscopic analyses. In this study, we now show that these PDF-induced morphological and cellular alterations of the peritoneal membrane are generally reversible after peritoneal rest.

The reduction of PDF-induced fibrosis appeared to be one of the significant consequences of peritoneal rest. It should be realized that decreased ECM thickening found after peritoneal rest is not due to diminished oedema, since the peritoneal fibrosis in this rat model is primarily because of the deposition of collagenous ECM, as we have shown previously by electron microscopy [7]. Our present finding is in good agreement with a previous work reporting diminished peritoneal fibrosis in rats exposed to PDF followed by 4 weeks' peritoneal rest [8]. Another *in vivo* study has shown that post-operative collagen synthesis in intestinal anastomoses is a rapid process and that it nearly returned to the pre-operative level after 4 weeks [11]. Complete regression of experimental vascular and glomerular sclerosis by angiotensin II-receptor blockade could also be achieved in a treatment period of only 4 weeks [12]. Furthermore, we show that peritoneal rest for 3 months resulted in blood vessel regression in the visceral peritoneum, especially in the mesentery, but not in the parietal peritoneum, indicating that blood vessel regression occurs with different kinetics in various peritoneal tissues. The present finding extends our previous observation that new vessel formation in various peritoneal tissues is also regulated differentially [7]. Mechanistically, blood vessel regression is associated with endothelial cell apoptosis [13]; thus, it is conceivable that the reduced vessel density upon peritoneal rest is due to programmed cell death. However, the PDF-induced angiogenesis was not fully normalized upon peritoneal rest; thus, most likely, more time is required for complete recovery. Our data indicate that the peritoneal rest-induced fibrotic resolution occurs faster than blood vessel regression; thus, the recovery of these major pathological changes happens with different kinetics. Understanding the mechanisms underlying fibrotic resolution and vessel regression might be of particular interest for possible therapeutic approaches.

In contrast to fibrosis and angiogenesis, the recovery of PDF-induced cellular changes of the peritoneal membrane occurs more rapidly, as almost all cellular parameters examined in this study were completely normalized after 3 months' peritoneal rest. For instance, PDF-induced mast cell response in the omentum returned to the control level after peritoneal rest. Likewise, mesothelial cells, which were damaged severely by daily exposure to PDF, again formed a good monolayer upon 3 months' peritoneal rest, as evidenced by electron microscopy. Our present finding appears to be inconsistent with an elegant study showing that after only 8 days free-floating mesothelial cells implant, proliferate and become incorporated into the reconstituted mesothelium after mesothelial injury *in vivo* [14].

Our present results show clearly that PDF-induced tissue remodelling is reversible, which might explain the improvement of the ultrafiltration found both in an animal PD model [8] and in PD patients [9,15] upon peritoneal rest. Although our data are obtained after 3 months' peritoneal rest that was preceded by only 5 weeks of peritoneal exposure in an animal model for PD, we suggest that peritoneal rest for a relatively short period of time might have interesting clinical consequences. This is of significant importance in cases where PD patients have to stop the treatment, for example when they switch to haemodialysis or when they undergo kidney transplantation.

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Conflict of interest statement. None declared.

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