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Peritoneal dialysis with solutions low in glucose degradation products is associated with improved biocompatibility profile towards peritoneal mesothelial cells

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Abstract

Background. *In vitro* experiments point to a better biocompatibility profile of new pH-neutral peritoneal dialysis fluids (PDFs) containing low levels of glucose degradation products (GDPs). The present study examines the impact on human peritoneal mesothelial cells (HPMCs) of equilibrated dialysates obtained during dialysis with either conventional or new PDFs.
Methods. Peritoneal dialysate was collected from 17 patients participating in a randomized, controlled, cross-over trial comparing a pH-neutral low-GDP solution (Balance) to a conventional solution (S-PDF). All patients were treated sequentially for 3 months with both PDFs. At the end of each treatment phase, peritoneal effluent was drained after a timed 10 h dwell. Samples of dialysate were then mixed with standard culture medium and added to *in vitro* cultures of HPMCs from healthy donors. Cells were assessed for proliferation, viability and cytokine release.
Results. Proliferation and viability of HPMCs were better preserved in the presence of effluent obtained during dialysis with Balance ($P < 0.046$ and $P < 0.035$, respectively). The proliferative response of HPMCs correlated with the concentration of fibronectin in dialysates ($P = 0.0024$). Effluent drained following a 3 month dialysis with Balance contained significantly increased levels of fibronectin ($P = 0.004$) and CA125 antigen ($P = 0.0004$) compared with S-PDF. There was no significant difference in constitutive and stimulated cytokine (IL-6, MCP-1, VEGF) synthesis by HPMCs treated with either Balance- or S-PDF-derived effluents.
Conclusions. These results suggest that therapy with new pH-neutral low-GDP solutions contribute to an intraperitoneal milieu that improves mesothelial cell proliferation and viability. It may positively impact on the preservation of the peritoneal membrane integrity during long-term dialysis.

Keywords: biocompatibility; glucose degradation products; peritoneal dialysis; peritoneal mesothelial cells

Introduction

It has recently been confirmed that peritoneal dialysis is associated with structural alterations in the peritoneum that may ultimately compromise its function as a dialysing membrane [1]. It is now believed that long-term exposure of the peritoneum to currently used peritoneal dialysis fluids (PDFs) contributes to the development of these changes. Over the past years many studies have demonstrated that PDFs exert adverse effects towards various populations of peritoneal cells *in vitro*. These studies have identified acidic pH, high concentration of glucose and lactate, increased osmolality and the presence of glucose degradation products (GDPs) as the main factors that limit biocompatibility of PDFs. For various reasons, however, it has proved difficult to replace currently used PDF components. Therefore,

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instead of changing PDF composition, it has been proposed to modify the process of its manufacturing. The low pH of conventional PDFs is necessary to prevent the caramelization of glucose during heat sterilization. The extent of acidification is, however, limited by biological and clinical tolerance. In conventional PDFs the pH is set to ~5.5, which does not seem to be low enough to eliminate glucose breakdown and formation of GDPs. As a consequence, in novel dual-chamber PDF systems highly concentrated glucose at pH as low as 2.8–3.2 is separated from an alkaline lactate solution. This design significantly reduces formation of GDPs during subsequent autoclaving of PDFs. Immediately prior to use the solutions from both compartments are mixed together and as result one receives a pH-neutral low-GDP solution to infuse into the peritoneal cavity.

The cytotoxic potential of GDPs present in PDFs has been demonstrated in several studies. We have recently observed significantly better preservation of viability and function in peritoneal mesothelial cells treated *in vitro* with new dual-chamber bag PDFs compared with conventional solutions of equal pH [2]. Indeed, by measuring heat shock protein expression, it has been confirmed that new generations of PDFs impose considerably less stress to mesothelial cells in culture [3].

Whilst these studies have shown differences in the response of mesothelial cells treated with unused PDF, it is not clear if such differences are still observable *in vivo*, where PDF is continuously being equilibrated. Following intraperitoneal instillation, the composition and properties of PDF change rapidly due to mixing with residual volume from preceding dwell and ultrafiltration from systemic circulation. It is well known that the low initial pH of PDF increases quickly, while concentrations of lactate and glucose decline with time. Moreover, it has been demonstrated that a substantial fraction of several GDPs present in PDF (glyoxal, methylglyoxal, 3-deoxyglucosone) is absorbed from the peritoneal cavity within a few hours [4]. As a result, the potential advantages of new PDFs, i.e. neutral pH and low GDP content, which are clearly seen *in vitro*, may in fact bear limited significance in the clinical setting.

In the present investigation, we have therefore assessed the function of peritoneal mesothelial cells treated with equilibrated dialysate effluent rather than by unused solutions. The samples of dialysate were obtained in the course of the Euro Balance Trial (EBT), a randomized, cross-over clinical study, during which patients were undergoing CAPD with the use of either conventional PDFs or a new pH-neutral low-GDP solution.

Subjects and methods

Patients

Twenty four patients recruited by four Polish centres participating in the multinational EBT were enrolled. All patients gave their informed consent and the local ethical committees approved the study protocol. All patients were using the stay·safe® system (Fresenius Medical Care, Bad Homburg, Germany). Twenty one patients completed the trial and of those, 17 had no record of peritonitis and/or exit site infection during 4 weeks prior to effluent collection. Data obtained from these patients were used for final comparisons between the dialysis solutions. Patient characteristics are presented in Table 1.

Table 1. Patient characteristics ($n = 17$)

Age (median and range) (years)	58 (25–79)
Gender (male/female)	10 (59%)/7 (41%)
Weight (median and range) (kg)	70.0 (43.2–97.8)
Height (median and range) (cm)	168 (150–176)
Primary renal disease	
Glomerulonephritis	4
Diabetic nephropathy	4
Polycystic kidney disease	2
Interstitial/obstructive	2
Unknown	5
Time on CAPD (median and range) (months)	7 (3–17)

Study design

The EBT was designed as a prospective, randomized, open, controlled, multi-centre and cross-over study, comparing a new dual-chambered solution with a conventional standard PDF (both manufactured by Fresenius Medical Care, and hereafter referred to as Balance and S-PDF, respectively) [Williams JD, Topley N, Craig KJ, Mackenzie RK, Pischetsrieder M, Lage C, Passlick-Deetjen J. The Euro-Balance Trial: The effect of a new biocompatible peritoneal dialysis fluid (balance) on the peritoneal membrane. Manuscript submitted for publication]. During an initial run-in phase of 4 weeks all patients received S-PDF according to their standard dialysis regimen. After that, they were randomized (1:1) to treatment with either S-PDF or Balance for 12 weeks (phase I). Then the two groups switched the solutions and were dialysed for a further 12 weeks (phase II). As a result, all patients were treated with both solutions over the course of the study.

Sample collection

At the end of treatment phases I and II, samples of peritoneal effluent were collected after a standardized overnight dwell of 10 h. The dwell was performed with a solution containing 2.3% glucose. Approximately 200 ml of effluent were withdrawn from the bag, filtered through a 0.2 μm pore size filter (Nalgene®; Nalge Nunc International, Rochester, NY, USA) to remove any cellular debris and aliquoted into polypropylene tubes (Sarstedt, Nümbrecht, Germany), centrifuged at 3000 *g* for 30 min and frozen at –70°C. After each treatment phase, all samples collected in participating centres were shipped in dry ice to the Department of Pathophysiology at Poznan Medical School for storage and analysis.

For initial experiments, samples of peritoneal effluent were obtained and pooled from eight stable CAPD patients not participating in the EBT. These samples were collected at specified times (15, 30 min, 1, 2, 4 h) after intraperitoneal infusion of conventional S-PDF containing 1.5% glucose.

Reagents

All chemicals, unless otherwise stated, were purchased from Sigma-Aldrich GmbH, Deisenhofen, Germany. All tissue culture plastics were obtained from Nunc, Roskilde, Denmark.

Human peritoneal mesothelial cells (HPMCs)

Cells were isolated, characterized and maintained in culture as described in detail elsewhere [5]. The standard culture medium was M199 supplemented with L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 µg/ml), hydrocortisone (0.4 µg/ml) and 10% (v/v) fetal calf serum (Gibco™; Invitrogen, Karlsruhe, Germany).

Exposure of HPMCs to peritoneal effluent

Cells were rendered quiescent by serum deprivation for 24 h. Samples of effluent were mixed with an equal volume (1:1) of standard serum-free medium M199 and then added to cell cultures. Following a defined exposure period, cells were tested for viability, proliferation, growth factor release and cytokine secretion, as described below.

Cell viability

Cell viability was measured with the MTT assay. Briefly, after exposure to effluent, cells were incubated in medium containing 1.25 mg/ml of the MTT salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolinum bromide) for 4 h at 37°C. The formazan product generated was solubilized by the addition of acidic solution of 20% (w/v) sodium dodecyl sulphate and 50% (v/v) *N*,*N*-dimethylformamide. Absorbance of the converted dye was recorded at 595 nm with a reference wavelength of 690 nm.

Cell proliferation

Proliferation of HPMCs was assessed by measuring incorporation of ^{3}H-thymidine into cultures during the exponential phase of growth. For these experiments, cells were plated onto 48 well clusters at density of $2.5 \times 10^{4}/cm^{2}$ and cultured for 24 h. The subconfluent HPMC cultures were then exposed to effluent being studied mixed with an equal volume of M199 serum-free culture medium labelled with ^{3}H-thymidine (used as methyl-^{3}H-thymidine at 1 µCi/ml; Institute of Radioisotopes, Prague, Czech Republic). After 24 h of incubation at 37°C, cells were detached with 0.05% trypsin/0.02% EDTA solution and precipitated with 10% (w/v) trichloroacetic acid. The precipitate was dissolved in 0.1 N NaOH and the released radioactivity was measured in a beta liquid scintillation counter (Wallac; Perkin Elmer, Warsaw, Poland).

Cytokine and growth factor synthesis

Quiescent HPMC monolayers were exposed to a mixture of effluent and medium and incubated for 24 h. In addition, selected HPMC cultures were stimulated with IL-1β (100 pg/ml). The post-culture supernates were centrifuged to remove any cellular debris and measured for IL-6, MCP-1 and VEGF, as described below.

Immunoassays

All immunoassays were performed using multi-well MaxiSorp test modules (Nunc). Immunoassays for IL-6, MCP-1 and VEGF were designed using ELISA-matched antibody pairs and performed according to the manufacturers' instructions. Antibodies against IL-6 and VEGF were purchased from R&D Systems Europe (Abingdon, UK), and those against MCP-1 from Pharmingen (BD Biosciences, Heidelberg, Germany). For determination of fibronectin in effluent anti-human fibronectin antibodies from Dako (Glostrup, Denmark) and Biogenesis (Quartett, Berlin, Germany) were used. The concentration of CA125 in effluent was measured using an electrochemiluminescence assay (ECLISA; Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. The sensitivity of the immunoassays was as follows: IL-6, 3 pg/ml; MCP-1, 5 pg/ml; VEGF, 10 pg/ml; fibronectin, 0.15 ng/ml; CA125, 0.6 U/ml. Since the concentrations of target molecules in samples often exceeded the range spanned by the standard curve, the samples were appropriately diluted with the assay diluent (150 mM NaCl, 20 mM Trizma base, 0.1% bovine serum albumin, 0.05% Tween-20, pH 7.3). Pilot experiments determined that at all dilutions used, the presence of effluent in samples being measured did not significantly interfere with the assays. Recovery of exogenous antigens added to diluted samples at concentrations within the range of standard curves were 96.7–114.1%. The background levels of cytokines and growth factors in effluent-containing media were always assayed in parallel and subtracted from the final results to assess specific mesothelial cell-derived cytokine and growth factor release. All results were normalized per 1 µg of cell protein, which was found to correspond to $2.1 \pm 1.0 \times 10^{3}$ cells (mean ± SD, $n = 16$). Protein concentrations in cell lysates and peritoneal effluent were estimated with the Bradford method using bovine serum albumin as a standard.

Statistical analysis

Each sample of peritoneal effluent was tested nine to 11 times with mesothelial cells isolated from separate, non-uraemic donors. A median value obtained from these experiments was taken for further analysis. Data from the two arms of the study were combined, so that comparisons were made between S-PDF and Balance irrespective of the order in which patients received these fluids. Since the run-in phase served mainly for standardizing the procedures and training, it was not included in the analysis, so that statistical comparisons were made only with the data obtained at the end of each treatment phase. The data were analysed with the Wilcoxon test for paired non-parametric data using GraphPad Prism™ 3.00 software (GraphPad Software Inc., San Diego, CA, USA). A P-value of < 0.05 was considered significant.

Results

Proliferation studies

Initial experiments have revealed that exposure of HPMCs to unused conventional S-PDF resulted in a significant inhibition of cell growth (Figure 1). In HPMCs incubated under these conditions, the

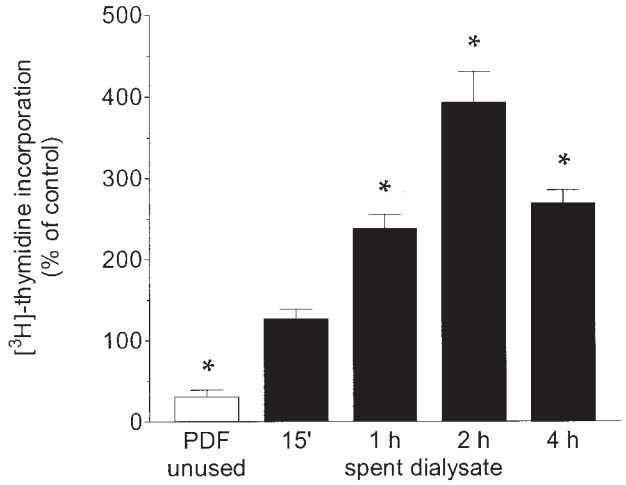

Fig. 1. Effect of peritoneal equilibration of PDF on mesothelial cell proliferation. HPMCs were exposed for 24 h to either unused conventional S-PDF or timed peritoneal effluent pooled from eight stable CAPD patients. Results are expressed as a percentage of ^{3}H-thymidine incorporation in control cells maintained in standard culture medium. Asterisks represent a statistically significant difference compared with the control.

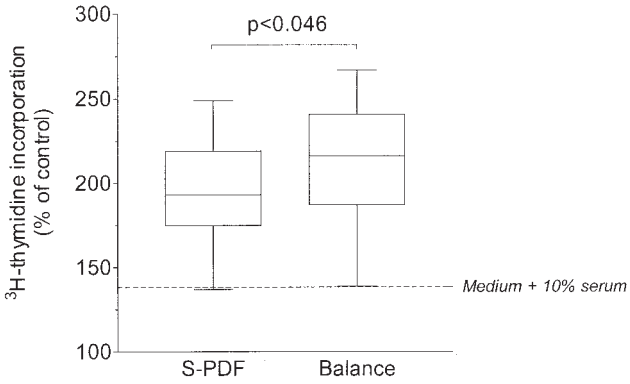

Fig. 2. Effect of peritoneal effluent on mesothelial cell proliferation. HPMCs were incubated for 24 h in ^{3}H-thymidine-labelled standard serum-free culture medium supplemented with an equal volume of spent dialysate. Timed 10 h effluent was obtained from patients ($n = 17$) after 3 months of CAPD with either Balance or S-PDF. Results are expressed as a percentage of ^{3}H-thymidine incorporation in control cells maintained in standard culture medium (100%). Box and whiskers plots represent the median, 25th and 75th percentiles, and the range of the data obtained from nine independent experiments.

incorporation of ^{3}H-thymidine was only 30.4 ± 8.7% (mean ± SD) of that observed in control cells. However, the dialysate effluent obtained after only 15 min of intraperitoneal dwell did not exhibit any inhibitory effects. In fact, *in vivo* equilibration for 1 h and longer resulted in effluents being capable of stimulating HPMC growth *in vitro*. The maximal stimulation was observed with a 2 h effluent and declined with samples drained at later time points.

Proliferation of HPMCs in the presence of a 10 h dwell effluent obtained when the patients had been dialysed with Balance PDF was 216.2 (138.7–266.9) (% of control; median and range) compared with 193.0 (136.8–248.8) ($n = 9$, $P < 0.046$) when they received conventional S-PDF (Figure 2). In cells cultured in the presence of 10% fetal calf serum ('positive' control), the incorporation of ^{3}H-thymidine was 138.9 ± 24.3% (mean ± SD) of that observed in control cells incubated in serum-free medium.

Since cell growth may be modulated by the concentration of protein in the incubation medium, we have related the rate of HPMC proliferation to the level of protein in effluents which HPMCs were exposed to. The non-parametric Spearman correlation coefficient for this relationship was found to be 0.2740 indicating a significant positive correlation at $P = 0.030$. More specifically, HPMC growth rate correlated significantly (Spearman $r = 0.3765$, $P = 0.024$) with the concentration of fibronectin in dialysate (Figure 3A). While there was no significant difference in total protein levels between Balance and S-PDF effluents (data not shown), measurements of fibronectin concentration revealed that dialysates drained during treatment with Balance contained significantly higher levels of fibronectin compared with the S-PDF effluent: 1495 (457–4487) *vs* 1144 (272–4122) (ng/ml; median and range) ($P < 0.0008$) (Figure 3B).

There was no significant correlation between the rate of HPMC growth and the concentration of CA125 in effluent (data not shown). However, the effluent levels of CA125 correlated significantly (Spearman $r = 0.4089$, $P = 0.0009$) with those of fibronectin (Figure 4A). Furthermore, the dialysate levels of CA125 were significantly higher in effluents obtained during dialysis with Balance compared with S-PDF (Figure 4B).

Mesothelial cell viability

As measured by the MTT assay, the viability of HPMCs exposed to Balance effluent was 0.729 (0.644–0.921) (OD; median and range) compared with 0.689 (0.648–0.819) in HPMCs exposed to S-PDF effluent ($P < 0.035$) (Figure 5). Both these levels were elevated compared with the average of control medium of 0.627.

Mesothelial cell cytokine and growth factor release

HPMCs exposed to the effluents produced comparable amounts of either IL-6, MCP-1 or VEGF, irrespective of the PDF type used (Table 2). The addition of IL-1β to effluents led to a synergistic induction of IL-6 and MCP-1. In this respect, there was a borderline difference between the groups with HPMCs exposed to Balance dialysate releasing slightly more IL-6 ($P < 0.057$).

Discussion

The design of studies assessing the toxicity of PDFs typically encompasses *in vitro* exposure to unused peritoneal dialysis solutions or selected fluid components, followed by the evaluation of defined cell functions. Such experiments are usually easy to perform,

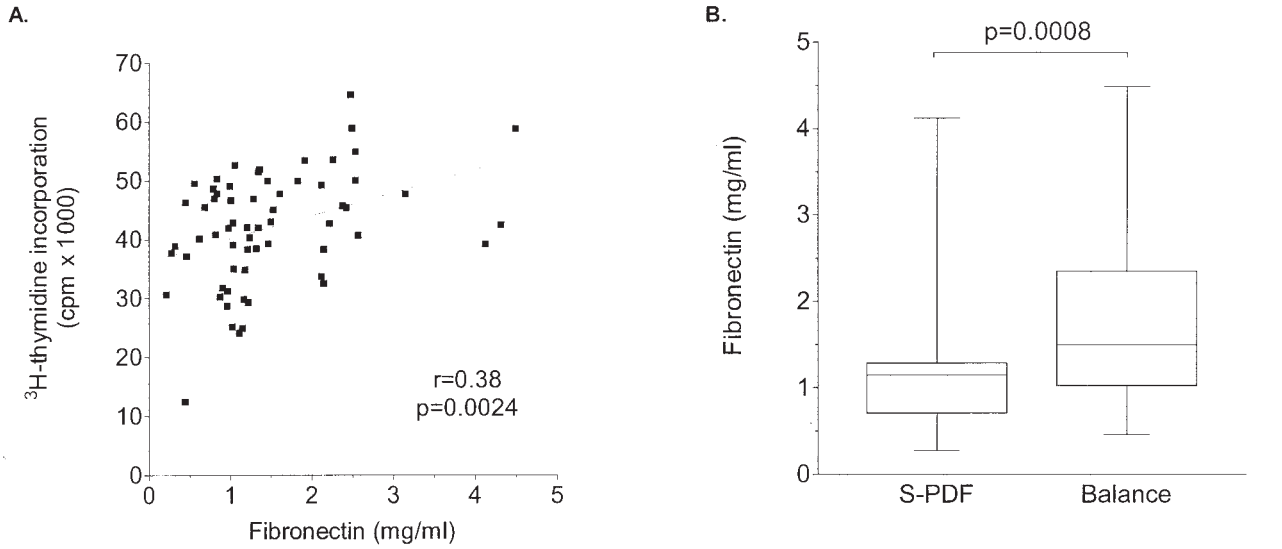

Fig. 3. (**A**) Correlation between mesothelial cell proliferation and the concentration of fibronectin in peritoneal effluent. A 24 h incorporation of ^{3}H-thymidine into cells exposed to samples of peritoneal effluent (obtained from EBT patients on 63 separate occasions) was related to the concentration of fibronectin in these samples. Results were derived from nine experiments with HPMCs from different donors. (**B**) Concentration of fibronectin in peritoneal effluent obtained from patients ($n = 17$) after 3 months of dialysis with either Balance or conventional S-PDF. Box and whiskers plots represent the median, 25th and 75th percentiles, and the range of the data.

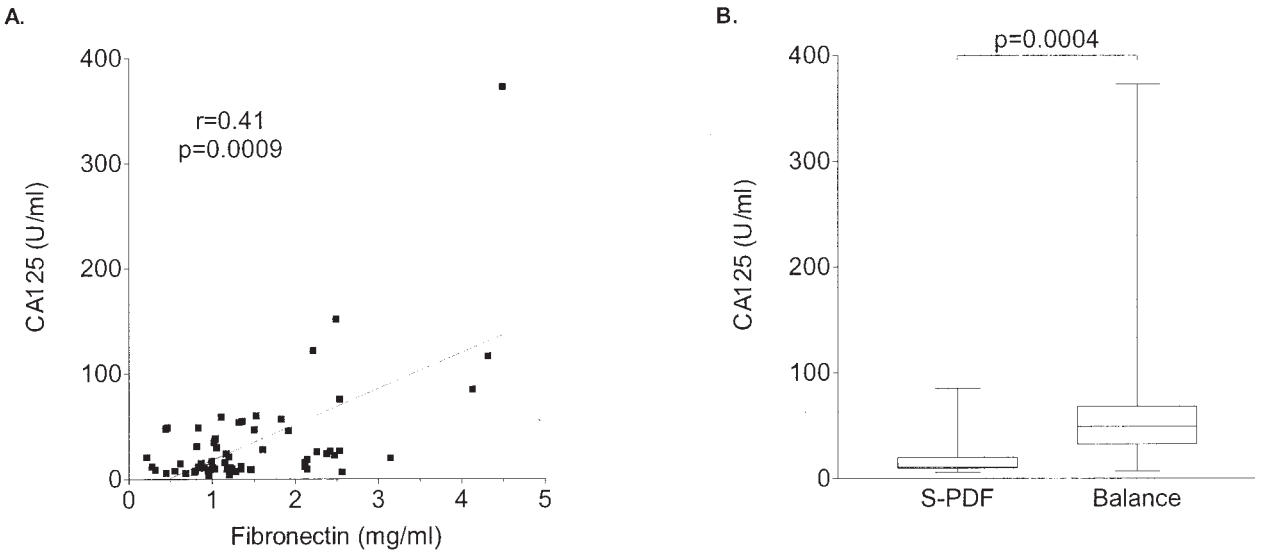

Fig. 4. (**A**) Correlation between fibronectin and CA125 levels in peritoneal effluent. Samples were obtained from EBT patients on 63 separate occasions. (**B**) Concentration of CA125 in peritoneal effluent obtained from patients ($n = 17$) after 3 months of dialysis with either Balance or conventional S-PDF. Box and whiskers plots represent the median, 25th and 75th percentiles, and the range of the data.

control and reproduce. In addition, interpretation of the results obtained is relatively straightforward. However, the major drawback of this method is that it does not really mimic the intraperitoneal milieu during CAPD. Therefore, pure *in vitro* settings are not able to offer full information on the *in vivo* impact of peritoneal dialysis solutions. For this reason, another approach aims at measuring dialysate levels of molecules that may reflect the status of the peritoneal membrane. Alas, so far no highly predictive and universal biocompatibility marker has been established. One may also try to isolate HPMCs from peritoneal effluent and assess their properties in subsequent culture *in vitro* [6]. However, this method requires large volumes of spent dialysate and its immediate processing after the drainage.

We have attempted to combine all these approaches by allowing the peritoneal dialysis solutions tested to equilibrate *in vivo*, by *in vitro* exposing peritoneal mesothelial cells to drained dialysates, and by relating observed cell responses to the levels of certain mediators in effluent. Since using such a protocol can generate the data that are influenced not only by potential differences between the solutions examined, but also by the inter-patient variability, and diverse responses of HPMCs prepared from different donors, the following precautions have been introduced.

To minimize differences originating from the patients' population (i) the cross-over design of the underlying clinical trial, (ii) parallel *in vitro* testing of samples from the same patient and (iii) the analysis by paired statistics were employed. The differences stemming from HPMC cultures were reduced by testing a given effluent sample with all cell lines and then by taking a median rather than a mean from these measurements as an average value representing this sample.

Our experiments have shown that the intraperitoneal dwell led to a rapid change in the properties of unused PDF, so that the effluent drained from the peritoneal cavity maintained rather than inhibited HPMC growth *in vitro*. The maximal proliferation stimulating activity was observed after 2 h of intraperitoneal dwell but then declined with time. We have chosen to examine an effluent from the overnight dwell as we believed it would represent a balanced mixture containing not only growth-promoting stimuli but also inhibitory factors.

Interestingly, the effect of spent dialysate on HPMC proliferation appeared to be greater with Balance than with S-PDF effluent. Although we have been able to analyse samples from only 17 patients, the significance of these results is increased by the fact that, in contrast to other studies assessing *ex vivo* function of peritoneal cells, the EBT was designed as a cross-over study in which each patient served as their own control. In addition, the viability of HPMCs *in vitro* seemed to be better preserved following treatment with Balance. Since the stimulatory activity of dialysate towards HPMC proliferation could be attributed to the gradual diffusion of plasma-derived components and/or the local release of growth factors, we have hypothesized that the difference in dialysate levels of these growth-promoting stimuli could explain the difference between the effects of Balance and S-PDF. Indeed, we have found that effluent drained during dialysis with Balance contained significantly higher concentrations of fibronectin compared with those obtained during dialysis with S-PDF.

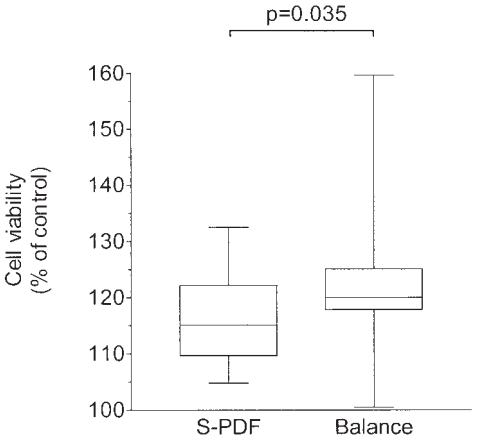

Fig. 5. Effect of peritoneal effluent on mesothelial cell viability. HPMCs were incubated for 24 h in standard serum-free culture medium supplemented with an equal volume of spent dialysate. Timed 10 h effluent was obtained from patients after 3 months of CAPD with either Balance or S-PDF ($n = 17$). Results are expressed as a percentage of values obtained in the MTT test for control cells maintained in standard culture medium (100%). Box and whiskers plots represent the median, 25th and 75th percentiles, and the range of the data obtained from four independent experiments with HPMCs obtained from different donors.

Fibronectin is an extracellular matrix protein that is known to stimulate mesothelial cell adhesion and proliferation [7]. The majority of fibronectin found in the peritoneal cavity is most probably of systemic origin [8]. It remains to be determined if Balance PDF may affect transperitoneal transport of fibronectin. On the other hand, although HPMCs do produce fibronectin, its fraction appears to be comparatively small. In fact we have been unable to accurately measure specific release of fibronectin from cells stimulated with effluent because of extremely high background (i.e. dialysate) levels of fibronectin compared with those secreted by HPMCs. However, in our previous study we have demonstrated that prolonged *in vitro* exposure of HPMCs to several identified GDPs resulted in a time-dependent loss of HPMC viability and, consequently, their ability to secrete fibronectin [9]. One may therefore imagine that dialysis with a low-GDP solution will help to maintain fibronectin synthesis in HPMCs and will enhance their proliferation *in vivo*. This view may be supported by changes in dialysate levels of CA125. Longitudinal changes in CA125 concentrations are believed to reflect mesothelial cell mass and viability [10]. We have found that intraperitoneal levels of CA125 were significantly increased when patients were receiving Balance compared to when they were treated with S-PDF. This observation concurs with previous reports of increased CA125 during dialysis with a new generation of dialysis fluids [11,12]. Although these solutions were based on

Table 2. Cytokine and growth factor release from HPMC exposed to effluent (median and range; statistical analysis S-PDF *vs* Balance)

Exposure	Mediator release (pg/μg cell protein)		
	IL-6	MCP-1	VEGF
Medium	1.4	6.2	0.3
S-PDF	3.7 (1.5–8.5)	32.2 (22.1–50.2)	6.8 (0.9–51.1)
Balance	4.2 (1.4–9.6); $P = 0.326$, NS	38.5 (20.6–63.3); $P = 0.197$, NS	8.7 (0.0–26.1); $P = 0.326$, NS
Medium + IL-1β	24.1	46.8	n.d.
S-PDF + IL-1β	39.2 (23.1–69.0)	158.4 (70.4–197.7)	n.d.
Balance + IL-1β	43.7 (31.0–87.0); $P = 0.057$, NS	151.6 (66.9–245.6); $P = 0.991$, NS	n.d.

different buffers (lactate or bicarbonate), they were all manufactured in multi-chamber systems and contained only trace amounts of GDPs. Interestingly, we have observed that increased levels of CA125 correlated significantly with higher concentrations of fibronectin but did not correlate directly with the *in vitro* growth of HPMCs in the presence of dialysate. It may indicate that while CA125 is a marker of mesothelial cell mass, it does not necessarily have to reflect cellular thymidine turnover measured over a short period of time.

The understanding of how peritoneal dialysis solutions affect HPMC growth *in vivo* appears to be of clinical significance. It has been demonstrated that the process of peritoneal dialysis induces continuous injury and regeneration in the peritoneal mesothelium [13]. Additional severe insult may be a consequence of peritonitis. Indeed, the Peritoneal Biopsy Registry® has documented loss of mesothelial lining in a significant number of patients undergoing CAPD [1]. Importantly, failure to re-mesothelialize is believed to be a prerequisite for subsequent development of peritoneal fibrosis and/or sclerosis that ultimately results in treatment failure [14].

The difference in HPMC growth *in vitro* in response to Balance and S-PSD effluents may appear small. However, the present study assessed proliferation of HPMCs only over a 24 h period. One may speculate that during long-term clinical CAPD with Balance these small differences may accumulate with time resulting in a peritoneal membrane covered with better preserved (or less injured) mesothelial cells. In this respect, recent data indicate that compared with conventional PDFs, new peritoneal dialysis solutions containing fewer GDPs improve mesothelial healing following *in vitro* wounding [15] and the process is associated with increased CA125 expression [16]. Furthermore, it is now known that GDPs accelerate the formation of advanced glycation end-products which are believed to contribute to peritoneal membrane dysfunction [4]. In this respect, it has been demonstrated that *in vitro* glycation of albumin in the presence of Balance PDF is significantly less pronounced compared with S-PDF [17]. More recently, reduced glycation in response to Balance PDF has been reported to also occur with collagen IV that forms the submesothelial basement membrane [18]. Interestingly, this Balance pre-exposed matrix has been found to better support subsequent growth of HPMCs *in vitro* compared with a template treated with S-PDF.

We have observed a similar release of cytokines from HPMCs incubated with test effluents. Additional stimulation with IL-1β resulted in a synergistic increase in IL-6 secretion which appeared to be marginally greater in cells treated with Balance. Increased levels of IL-6 in dialysate have been recently shown to correlate significantly with dialysate VEGF, and both mediators were linked with increased peritoneal permeability [19]. While this association raises some concerns, we have found no evidence of increased VEGF release from cells exposed to Balance-derived effluent over the period of 24 h. On the other hand, recent data point to a crucial role of IL-6 in coordinating the inflammatory response [20]. In this respect, the readiness of cells to mount IL-6 may be viewed as promoting the prompt resolution of inflammation.

Taken together, our data suggest that dialysis with a new pH-neutral low-GDP solution is associated with improved proliferative response of peritoneal mesothelial cells, at least from non-uraemic patients. This impact may help to maintain integrity of the peritoneal membrane during long-term peritoneal dialysis.

Conflict of interest statement. None declared.

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