

Original Article

Effect of lactate and bicarbonate on human peritoneal mesothelial cells, fibroblasts and vascular endothelial cells, and the role of basic fibroblast growth factor

Satoshi Ogata¹, Takayuki Naito^{1,2}, Noriaki Yorioka², Kei Kiribayashi², Masatoshi Kuratsune² and Nobuoki Kohno²

¹Department of Internal Medicine, National Kure Medical Center, Kure and ²Department of Molecular and Internal Medicine, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan

Abstract

Background. In patients on long-term continuous ambulatory peritoneal dialysis (CAPD), peritoneal dysfunction may occur due to loss of peritoneal mesothelial cells, peritoneal fibrosis and neovascularization. Lactate, long used as a buffer in peritoneal dialysates, has been substituted by bicarbonate in recent years. However, their effects on the peritoneum of CAPD patients are unknown. This study investigated the influence of lactate and bicarbonate on peritoneal dysfunction in CAPD patients.

Methods. The mitochondrial activity of human peritoneal mesothelial cells (HPMCs) and their expression of basic fibroblast growth factor (bFGF) were studied after culture under various conditions. We also assessed the mitochondrial-activating effect of the supernatant of those cultures on human peritoneal fibroblasts (HPFBs) and human umbilical vein endothelial cells (HUVECs) and the effect of recombinant human bFGF on the mitochondrial activity of HPFBs and HUVECs. We used the WST-1 assay to determine mitochondrial activity in HPMC.

Results. At pH 7.4, the mitochondrial activity of HPMCs was lowest in a medium containing 40 mM (Lac), intermediate in a lactate (15 mM) plus bicarbonate (25 mM) medium (Lac/Bic), and highest in a 40 mM bicarbonate medium (Bic). In culture supernatant, the increase of bFGF was: Lac > Lac/Bic > Bic. Mitochondrial activation of HPFBs and HUVECs was stimulated by HPMC culture supernatants in the following decreasing order: Lac > Lac/Bic > Bic. The effects of these supernatants were suppressed by a bFGF-neutralizing antibody, while recombinant bFGF

caused concentration-dependent mitochondrial activation in HPFBs and HUVECs.

Conclusions. The role of bFGF in peritoneal fibrosis and neovascularization may be important. A bicarbonate-containing medium is better than a lactate-containing medium for preserving cell viability in HPMCs and preventing bFGF expression by these cells.

Keywords: fibrosis; growth factors; peritoneal dialysis; vascular reactivity

Introduction

Continuous ambulatory peritoneal dialysis (CAPD) has been used for two decades to treat end-stage renal failure, but peritoneal dysfunction still is an important problem. Loss of peritoneal mesothelial cells, peritoneal fibrosis and neovascularization of the peritoneum gradually progress in almost all patients on CAPD; and these changes are suspected to have a relationship to the loss of peritoneal function [1,2]. Recently, Murata *et al.* demonstrated that increased transperitoneal solute transport (for both creatinine and β_2 -microglobulin) is associated with the increase in the surface area of peritoneal microvessels, especially in patients on long-term CAPD [3]; however, the mechanisms involved in the initiation and progression of peritoneal dysfunction remain to be elucidated.

In CAPD patients, the peritoneal mesothelium acts as a protective barrier; it is also involved in the transport of water and solutes, remodelling after injury and production of many biologically active substances—including cytokines, growth factors and adhesion molecules. Since unphysiological peritoneal dialysates could damage mesothelial cells, mesothelial

Correspondence and offprint requests to: Dr N. Yorioka, Department of Molecular and Internal Medicine, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan.
Email: nyorioka@hiroshima-u.ac.jp

injury has been suggested as the primary reason for peritoneal dysfunction in patients on CAPD.

Lactate has long been used as a buffer in peritoneal dialysates, but bicarbonate has been substituted for it in recent years. Lactate-containing CAPD solutions induce greater increases of microvascular flow and perfused capillary length per area of peritoneal membrane than bicarbonate solutions, and they cause a more marked decrease of human neutrophil function [4]; however, the effects of lactate and bicarbonate on the peritoneum in CAPD patients are still unknown.

Basic fibroblast growth factor (bFGF) is a classical cytokine, along with platelet-derived growth factor and transforming growth factor- β (TGF- β). It is known to induce fibroblast proliferation and thus to participate in the development of many fibrotic diseases, including glomerulonephritis, prostatic hyperplasia, chronic liver disease and pulmonary fibrosis. It also participates in many diseases related to angiogenesis (e.g. diabetes mellitus, Kaposi's sarcoma, myelofibrosis and cutaneous haemangioma) by inducing the proliferation of endothelial and vascular smooth muscle cells, as well as the production of vascular endothelial growth factor (VEGF), which has an important role in the development of peritoneal neovascularization and a close relationship with bFGF [5,6]. Although bFGF has been detected in peritoneal dialysis effluent [7], its role in peritoneal neovascularization has been poorly documented. It was reported previously that recombinant bFGF, peritoneal dialysis effluent and the conditioned medium of cultured human peritoneal mesothelial cells (HPMCs) all have a mitogenic effect on peritoneal fibroblasts, while the proliferative effect of the dialysis effluent is reduced by co-incubation with an anti-bFGF antibody [8]. We previously showed that culturing HPMCs in a high-glucose medium caused an increase of bFGF expression, that the secretion of bFGF by HPMCs can stimulate the proliferation of human peritoneal fibroblasts (HPFBs) and that bFGF can also promote the secretion of fibronectin by HPFBs [9].

In order to clarify the *in vitro* mechanism of peritoneal fibrosis and neovascularization in the presence of lactate or bicarbonate, we investigated the mitochondrial activity of HPMCs and the expression of bFGF by them after culture in media containing lactate or bicarbonate, or both. We also investigated whether or not the release of bFGF by HPMCs could cause mitochondrial activation in HPFBs and human vascular endothelial cells. We used the WST-1 assay to determine mitochondrial activity in HPMCs, whose increase is an indirect indication of robust cell viability.

Materials and methods

Preparation of cells

HPMCs and HPFBs were isolated according to the methods of Stylianou *et al.* [10] and Beavis *et al.* [8], respectively.

To obtain HPMCs, pieces of human omentum (3–5 cm²) were harvested at laparotomy from non-uraemic and non-diabetic patients who were not on dialysis and who had gastric cancer without metastasis (tissue samples were obtained from >30 donors). The tissues were washed three times with sterile phosphate-buffered saline (PBS; pH 7.3; Nissui Pharmaceutical Co., Tokyo, Japan), then incubated with shaking at 37°C in 0.125% (w/v) trypsin–0.01% (w/v) EDTA solution (Life Technologies, Grand Island, NY) for 10 min.

To isolate HPFBs, omental tissue that had been processed to remove mesothelial cells was incubated in the trypsin–EDTA solution at 37°C with continuous rotation. Incubation was performed twice for 40 min each, with fresh trypsin–EDTA used for the second incubation.

Next, the residual tissue was removed, and the trypsin solution containing free mesothelial cells or fibroblasts was centrifuged at 100 g for 10 min at 4°C. The supernatant was discarded, and the cell pellet was washed once with PBS. Then, the pellet was suspended in an M199 medium supplemented with 10% fetal calf serum (FCS; v/v) (Mitsubishi Kasei Corp., Tokyo, Japan), 100 U/ml penicillin (Life Technologies), 100 µg/ml streptomycin (Life Technologies) and 2 mM L-glutamine (Life Technologies).

Human umbilical vein endothelial cells (HUVECs) were obtained from the American Type Culture Collection (Rockville, MD). We used HUVECs instead of peritoneal vascular cells, because a method for the isolation and culture of human peritoneal vascular cells has not yet been developed.

After being seeded into 75 cm² (250 cm³) tissue culture flasks coated with type I rat collagen (Becton Dickinson, Bedford, MA), the cells were maintained in the same medium and incubated at 37°C under an atmosphere of 5% CO₂/95% air in a humidified incubator. The medium was replaced every 2–3 days.

The harvesting of the samples of omentum was approved by the medical ethics committee of Hiroshima University Graduate School of Biomedical Sciences, and informed consent was obtained from each patient. We used samples of omentum from at least three different donors in each experiment, and all experiments were repeated in triplicate using cells from those same donors. HPMCs from the first or second passages, HPFBs from the third to fifth passages and HUVECs from the tenth to fifteenth passages were used for these experiments.

Identification of mesothelial cells and fibroblasts

Cultured cells were examined under an inverted-phase-contrast microscope. In addition, immunostaining was performed with monoclonal antibodies for human cytokeratin (an epithelial and mesothelial cell marker; Dako, Kyoto, Japan), vimentin (a myogenic cell marker; Dako) and human factor VIII (an endothelial cell marker; Dako). Visualization was done by using a rhodamine-conjugated monoclonal secondary antibody (Jackson Immuno Research Laboratories, West Grove, PA) for the detection of fluorescence.

Culture media

This medium was supplemented further with sodium lactate (Katayama Chem. Inc., Osaka, Japan), sodium bicarbonate

(Katayama Chem. Inc.) or both. Either lactate or bicarbonate was added to a concentration of 40 mM, while the concentration of the combined medium (Lac/Bic medium) was 15 mM for lactate and 25 mM for bicarbonate. All media were sterilized by filtration (0.22 μ m, Millex-GV, Millipore, Bedford, MA) and buffered just before use to a pH of 7.4 with 1 M sodium hydroxide (Katayama Chem. Inc.) or 1 M hydrochloric acid (Katayama Chem. Inc.) (Table 1).

WST-1 assay

Mitochondrial activity was assessed using a colorimetric assay kit (Dojindo, Kumamoto, Japan), which was based on cleavage in viable cells of the water-soluble tetrazolium salt WST-1 [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfonyl)-2H-tetrazolium, monosodium salt] to a formazan dye by mitochondrial dehydrogenase. We used the WST-1 assay to determine mitochondrial activity in HPMCs; an increase in this activity is an indirect indication of cell viability. Culture media were added (200 μ l/well) to quiescent HPFBs and HUVECs in 96-well plates, and incubation was performed, with the WST-1 solution (5 mM WST-1, 20 mM HEPES and 0.2 mM 1-methoxy-5-methylphenazinium methylsulfate) being added at 10 μ l/well for the last 4 h. Formazan dye absorbance was determined using an enzyme-linked immunosorbent assay (ELISA) reader (SLT Lab Instruments, Salzburg, Austria) at a reading wavelength of 420 nm and a reference wavelength of 630 nm. The data were corrected according to the inter- and intra-assay coefficients of variation.

Harvesting culture supernatants and measuring bFGF

Supernatants were harvested after mesothelial cells (1×10^5 /well) had been cultured for 24 h with various media (1.0 ml/well) in 24-well dishes coated with type I rat collagen (Becton Dickinson), and were stored at -30°C until assay. The bFGF level in the culture supernatants was measured with a sandwich ELISA kit (R&D Systems Inc., Minneapolis, MN).

Activation of HPFBs and HUVECs by HPMC supernatant

The activating effect of the supernatant from HPMCs on mitochondria from HPFBs and HUVECs was assessed by the WST-1 assay mentioned above. Supernatants [with or without a bFGF neutralizing antibody (0.1, 1 or 10 μ g/ml, Wako, Osaka, Japan) or mouse IgG (10 μ g/ml, Sigma)] were

added at 200 μ l/well to quiescent HPFBs (5×10^3 /well) and HUVECs (1×10^4 /well) in 96-well plates, and incubation was done for 48 h, the WST-1 solution being added for the last 4 h at 10 μ l/well.

Effect of recombinant bFGF on HPFBs and HUVECs

The activating effect of recombinant bFGF on mitochondria of HPFBs and HUVECs was also assessed by the WST-1 assay. Culture media [M199 medium with 0.1% (HPFBs) or 1% (HUVECs) FCS, 0–1000 pg/ml of recombinant bFGF (Pepro Tech Inc., Rocky Hill, NJ), and 0 or 0.5 μ g/ml of bFGF neutralizing antibody] were added at 200 μ l/well to quiescent HPFBs (5×10^3 /well) and HUVECs (1×10^4 /well) in 96-well plates, and incubation was done for 48 h, the WST-1 solution being added for the last 4 h at 10 μ l/well.

Statistical analysis

Statistical analysis was performed using the unpaired Student's *t*-test or the Mann-Whitney U-test as appropriate. Results are expressed as the mean \pm SEM, and *P*-values of <0.05 were considered statistically significant.

Results

Identification of HPMCs and HPFBs

Cultured cells were identified as mesothelial cells by their typical cobblestone appearance at confluence, as well as by positive immunostaining for cytokeratin (Figure 1A) and vimentin, plus negative staining for factor VIII. Fibroblasts were identified by their

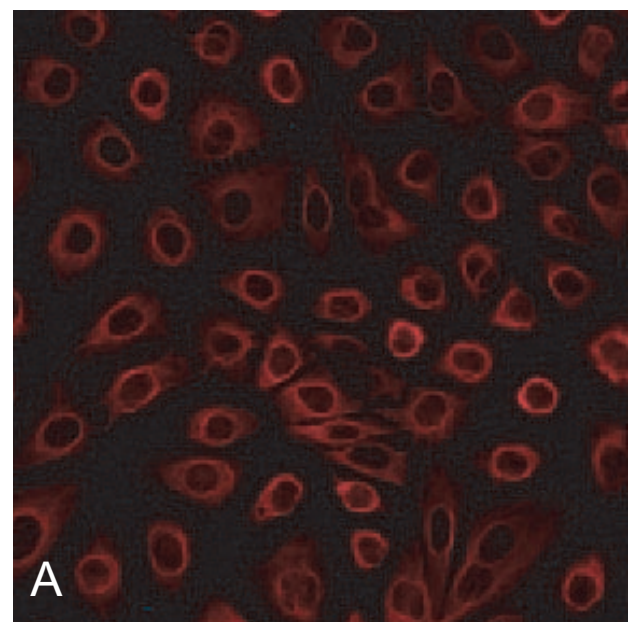


Fig. 1. Photomicrographs of human peritoneal mesothelial cells (A) and human peritoneal fibroblasts (B), both immunostained for cytokeratin using a rhodamine-conjugated secondary monoclonal antibody (magnification, $\times 400$).

Table 1. Composition of the test media

	Lac	Lac/Bic	Bic
Basal medium (standard M199 medium without Bic)			
FCS	10	10	10
pH	7.4	7.4	7.4
Lactate (mM)	40	15	0
Bicarbonate (mM)	0	25	40

Lac = sodium lactate; Bic = sodium bicarbonate; FCS = fetal calf serum.

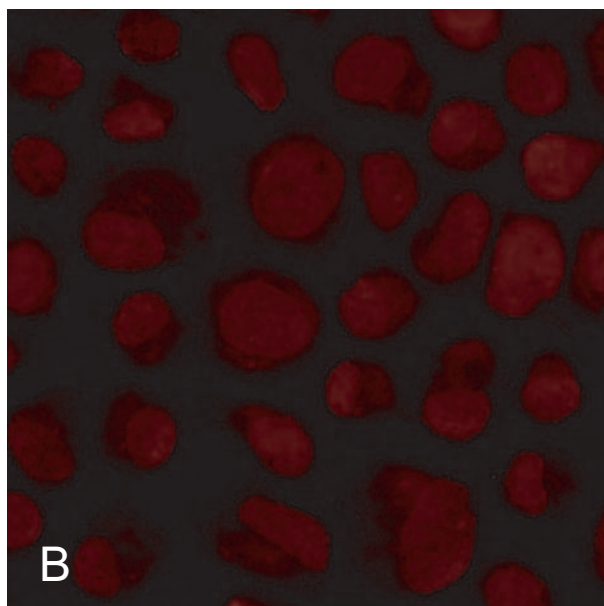


Fig. 1. Continued.

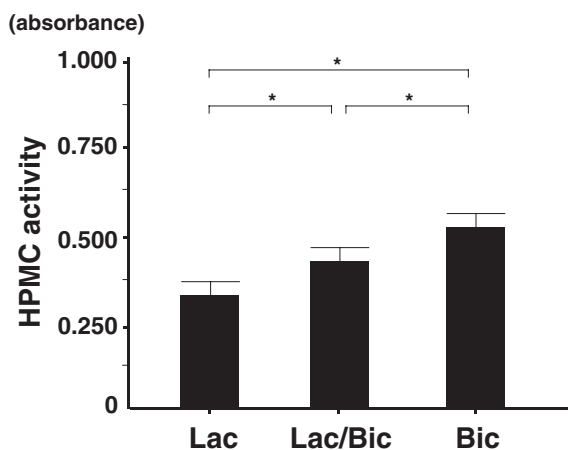


Fig. 2. Mitochondrial activity of human peritoneal mesothelial cells (HPMCs) after incubation with a medium at pH 7.4 containing 40 mM lactate (Lac), 15 mM lactate + 25 mM bicarbonate (Lac/Bic) or 40 mM bicarbonate (Bic). The response was assessed by the WST-1 assay after incubation for 24 h. Results are shown as the mean ± SEM of three experiments, with each being performed in triplicate. * $P < 0.05$.

typical bipolar and multipolar morphology along with positive immunostaining for vimentin and negative staining for cytokeratin (Figure 1B) and factor VIII. Cultures of a specific cell type were excluded if contaminated by other cell types.

Activity of HPMCs

The mitochondrial activity of HPMCs after incubation at pH 7.4 for 24 h was lowest in the 40 mM lactate medium (0.338 ± 0.031), intermediate in the Lac/Bic medium (0.426 ± 0.024) and highest in the 40 mM bicarbonate medium (0.552 ± 0.027) (Figure 2).

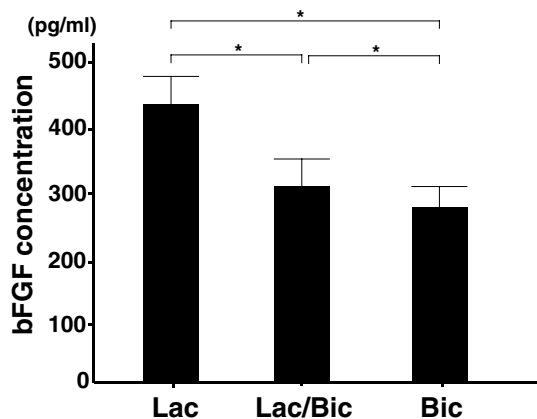


Fig. 3. Basic fibroblast growth factor (bFGF) concentrations in the culture supernatant of human peritoneal mesothelial cells after incubation with media at a pH of 7.4 containing 40 mM lactate (Lac), 15 mM lactate + 25 mM bicarbonate (Lac/Bic) or 40 mM bicarbonate (Bic). bFGF was measured by ELISA after the incubation of cells for 24 h. Results are shown as the mean ± SEM of three experiments, with each being performed in triplicate. * $P < 0.05$.

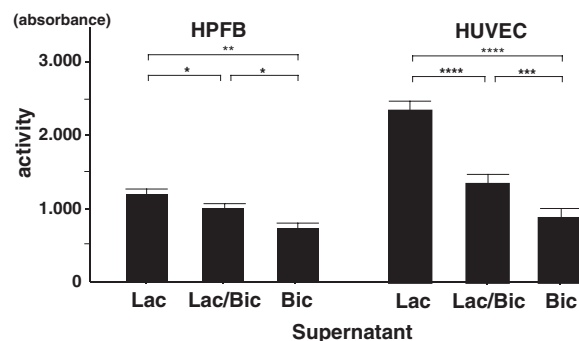


Fig. 4. Activation of human peritoneal fibroblasts (HPFBs) and human umbilical vein endothelial cells (HUVECs) by the supernatants of the cultures of human peritoneal mesothelial cells incubated in the presence of 40 mM lactate (Lac), 15 mM lactate + 25 mM bicarbonate (Lac/Bic) or 40 mM bicarbonate (Bic). The response was assessed by the WST-1 assay after incubation for 48 h. Results are shown as the mean ± SEM of three experiments, with each being performed in triplicate. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

bFGF secretion by HPMCs

The maximum difference between lactate- and bicarbonate-containing media was found at 24 h in experiments on the time course of bFGF protein secretion (data not shown). Confluent HPMCs secreted bFGF, with its concentrations in the culture supernatants falling in the following order after 24 h of incubation at pH 7.4: in the 40 mM lactate medium (441 ± 39 pg/ml) > in the Lac/Bic medium (302 ± 39 pg/ml) > in the 40 mM bicarbonate medium (275 ± 33 pg/ml) (Figure 3).

Activation of HPFBs and HUVECs

The mitochondrial activity of HPFBs and HUVECs was stimulated by the culture supernatants in the

following order of decreasing magnitude: the 40 mM lactate medium (HPFBs, 1.221 ± 0.023 ; HUVECs, 2.398 ± 0.031) > the Lac/Bic medium (HPFBs, 1.066 ± 0.017 ; HUVECs, 1.331 ± 0.039) > the 40 mM bicarbonate medium (HPFBs, 0.777 ± 0.008 ; HUVECs, 0.884 ± 0.044) (Figure 4). The effect of lactate-containing supernatants was suppressed by blocking bFGF with a bFGF-neutralizing antibody (HPFBs, 10 mg/ml, 0.508 ± 0.011 ; HUVECs, 10 mg/ml, 1.284 ± 0.042), but not by mouse IgG (10 mg/ml, HPFBs, 1.302 ± 0.045 ; HUVECs, 2.626 ± 0.040) (Figure 5). Recombinant human bFGF caused concentration-dependent mitochondrial activation in both HPFBs and HUVECs (HPFBs, 0 pg/ml, 0.383 ± 0.050 , 1000 pg/ml, 0.615 ± 0.039 ; HUVECs, 0 pg/ml, 1.002 ± 0.026 , 1000 pg/ml,

1.309 ± 0.032), but this effect was prevented by the bFGF-neutralizing antibody (0.5 mg/ml, HPFBs, 0.312 ± 0.026 ; HUVECs, 0.996 ± 0.020) (Figure 6).

Discussion

This study demonstrated *in vitro* effects of bFGF that suggest it may have an important role in the development of peritoneal fibrosis and neovascularization in CAPD patients. It also showed that a bicarbonate-containing medium is better than a lactate-containing one for preserving HPMC activity and for preventing bFGF production by HPMCs, and thus possibly for suppressing the activation of HPFBs and HUVECs.

The mitochondrial activity of HPMCs incubated for 24 h at pH 7.4 showed a decrease in the lactate-containing medium, but not in the bicarbonate-containing medium. This might indicate that the loss of HPMCs was prevented by the medium containing bicarbonate rather than lactate. Plum *et al.* [11] demonstrated that bicarbonate solutions were superior to lactate solutions (both adjusted to physiological pH) with respect to their effects on MTT assays and intracellular ATP levels, and our results support their data.

The data on bFGF secretion were not corrected for changes in the activity of HPMCs. However, our results indicate that the total bFGF secreted by HPMCs increased, despite the impaired activity of these cells in the lactate-containing medium. It seems that 'individual' cells secreted extremely high amounts of bFGF in spite of their loss of mitochondrial activity.

The concentration of bFGF in the conditioned medium of the HPMCs incubated with lactate was ~ 440 pg/ml by ELISA. The supernatant from incubation with this medium increased mitochondrial activity in HPFBs and HUVECs by ~ 1.6 and 2.7 -fold, respectively; and this activation was markedly suppressed by a bFGF-neutralizing antibody. This effect suggests that bFGF secreted by HPMCs may have an important role in the development of fibrosis and neovascularization of the peritoneum. On the other hand, recombinant bFGF had a weak effect on HPFBs and HUVECs, which might have been related to the FCS concentration; but it is also possible that other cytokines or growth factors, etc., influence bFGF activity. The action of bFGF on HPMCs and HUVEC may be via other factors in serum, or it may enhance the secretion of other factors by HPMCs incubated with lactate.

In addition, as shown in Figure 6, the activities of HPFBs or HUVECs incubated with bFGF-neutralizing antibody were lower than that of control cells. Cronauer *et al.* [12] reported that almost 80% of bFGF was intracellular, $\sim 20\%$ was associated with extracellular matrix components on the surface of HPMCs, and that a small amount was detectable in culture supernatants. Accordingly, some bFGF might be

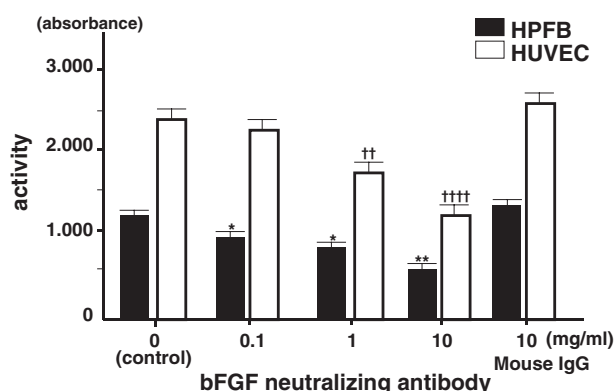


Fig. 5. The effect of a bFGF neutralizing antibody on the response of human peritoneal fibroblasts (HPFBs) and human umbilical vein endothelial cells (HUVECs) to the culture supernatant from human peritoneal mesothelial cells incubated with 40 mM lactate. Cell responses were assessed by the WST-1 assay after incubation for 48 h. Mouse IgG was used as a control. Results are shown as the mean \pm SEM of three experiments, with each being performed in triplicate. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ vs control.

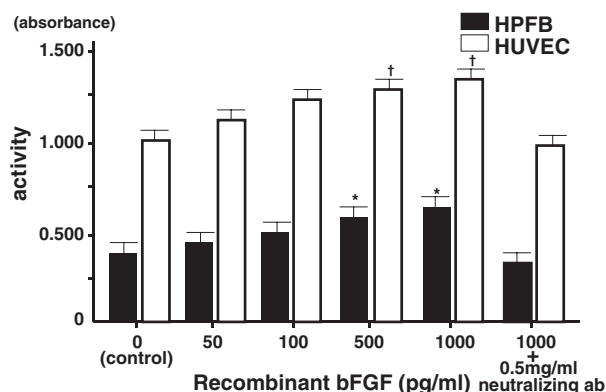


Fig. 6. The effect of recombinant bFGF on the mitochondrial activity of human peritoneal fibroblasts (HPFBs) and human umbilical vein endothelial cells (HUVECs). Activity was assessed by the WST-1 assay after incubation for 24 h. bFGF-neutralizing antibody was also added as a control. Results are shown as the mean \pm SEM of three experiments, with each being performed in triplicate. * $P < 0.05$, † $P < 0.05$ vs control.

secreted by HPFBs or HUVECs, and/or bFGF neutralizing antibody might inhibit the autocrine effect of intracellular bFGF in these cells.

In this study, we found statistically significant differences ($P < 0.05$), but they may not be of biological significance. However, we performed most experiments over a period of 24–48 h and obtained significant results in such a short time frame. Accordingly, these differences might become more important in patients on chronic CAPD.

VEGF is an endothelial-specific growth factor that potently stimulates an increase of microvascular permeability and proliferation, as does bFGF. VEGF is mainly localized in the mesothelial layer and vessel walls of the peritoneum in patients on chronic CAPD [6]. High glucose levels upregulate VEGF expression in various types of cells and tissues. De Vriese *et al.* [13] showed that the induction of structural and functional microvascular alterations by hyperglycaemia could be prevented by long-term treatment with a neutralizing anti-VEGF monoclonal antibody. VEGF may be essential for functional changes of the peritoneal membrane, but there is little evidence that VEGF itself has a fibrogenic effect and causes peritoneal fibrosis. In fact, VEGF administration reportedly reduces renal fibrosis and stabilizes renal function [14]. Thus, there is no consensus about the role of VEGF in fibrotic diseases. Accordingly, in the present study, we concentrated on investigating the role of bFGF, which has both angiogenic and fibrogenic effects.

Several reports have suggested the importance of TGF- β in the mechanism of peritoneal fibrosis, but recent studies have shown that FGF enhances the proliferation of cultured HPFBs and has a far stronger effect than TGF- β . Beavis *et al.* [8] and Fukasawa *et al.* [15] reported that recombinant bFGF significantly promoted the proliferation of peritoneal fibroblasts, whereas recombinant TGF- β suppressed or did not influence the proliferation of these cells. Accordingly, we investigated the role, not of TGF- β , but of bFGF in peritoneal fibrosis, and we obtained findings supporting these reports.

Dialysates containing lactate at a concentration of 40 mM, bicarbonate at concentrations of 34 and 39 mM, or a combination of 15 mM lactate and 25 mM bicarbonate are currently available for clinical use. We adopted a total concentration of 40 mM so that we could investigate the effects of lactate and bicarbonate at the same concentration. There was no control (for neither lactate nor bicarbonate) because dialysates must contain some buffer. There have been a few reports about the *in vitro* effects of lactate and bicarbonate on human peritoneal mesothelial cells [4,11], but the effects of these buffers on the expression of bFGF by peritoneal mesothelial cells have not been investigated.

There have been several reports on clinical experience with bicarbonate- and lactate-buffered peritoneal dialysis solutions. Feriani *et al.* [16,17] showed that using bicarbonate-buffered dialysate for CAPD

improved acid–base status, replenished bicarbonate stores and increased the normalized rate of protein catabolism when compared with lactate-buffered dialysate. Feriani *et al.* also showed that undesirable metabolic alkalosis could be avoided by adjusting the concentration of bicarbonate based on the level of acid production [16].

Acids that form in the body during metabolism are neutralized by various intrinsic buffer systems, of which bicarbonate is the most important (because the human body does not use lactate as a buffer). Lactate is metabolized in the liver, so patients with hepatic dysfunction (e.g. cirrhosis, severe heart disease, neonates) have a high risk of hyperlactacidaemia. Therefore, patients (especially children with end-stage renal disease) who may not be able to metabolize lactate well should be dialysed with a pure bicarbonate solution. Because the usual lactate concentration in humans is ~ 2 mM, it is reasonable to adopt a lactate concentration lower than 2 mM for CAPD solutions to avoid diffusion of lactate from the dialysate to the patient.

Unphysiological CAPD fluids (with high glucose content, low pH, added lactate and hyperosmolarity) have been used for many years. In an attempt to make CAPD fluids more physiological, various improvements have been made, such as the addition of icodextrin and sodium bicarbonate or neutral pH fluids and the dual-chambered bag system. The new neutral pH dialysis fluid may preserve peritoneal cell viability and function, and may decrease the production of glucose degradation products and advanced glycation end-products, which show strong cytotoxicity and a fibrogenic effect [18]. The normal pH of the human body is ~ 7.4 . Since in the near future all CAPD fluids may be set at this physiological pH, in the present study we investigated the effect of lactate and bicarbonate at a pH of 7.4.

However, Liberek *et al.* [19] reported that neither lactate nor a low pH caused significant suppression of leukocyte function, so there is no consensus about the effects of lactate and pH on peritoneal cells.

In conclusion, the present study demonstrated a possible role for bFGF in peritoneal fibrosis and neovascularization related to the use of lactate- or bicarbonate-containing dialysates for CAPD; but the actual effects of bFGF in CAPD patients remain to be elucidated. Our *in vitro* data suggest that lactate-containing CAPD solutions may be more likely to induce fibrosis and neovascularization than bicarbonate-containing solutions, and they support the superiority of a pure bicarbonate solution. However, cellular activity and release of growth factors may differ between the uraemic and non-uraemic states. Plum *et al.* [20] showed that peritoneal mesothelial cells from uraemic patients release interleukin (IL)-8 after a milder stimulation with IL-1 β than cells from healthy donors. Further investigations are required to determine the ideal dialysate for CAPD patients.

Acknowledgements. The authors wish to thank Drs Shirou Nakai, Mikio Fujimoto, Takahiro Santo, Katsunari Miyamoto, Toru Ogata and Jun Kawamoto (Department of Surgery, Hiroshima Memorial Hospital) for their cooperation in collecting samples during abdominal surgery, and Professor Jose A. Diaz-Buxo (Clinical Professor of Medicine, University of North Carolina) for consultation on the clinical use of bicarbonate dialysate. A portion of this paper was presented in abstract form at the XXXIX Congress of the European Renal Association-European Dialysis and Transplant Association, July 2002 and awarded 'Poster of Excellence'.

Conflict of interest statement. None declared.

References

- Mateijsen MA, van der Wal AC, Hendriks PM *et al.* Vascular and interstitial changes in the peritoneum of CAPD patients with peritoneal sclerosis. *Perit Dial Int* 1999; 19: 517–525
- Williams JD, Craig KJ, Topley N *et al.* Morphologic changes in the peritoneal membrane of patients with renal disease. *J Am Soc Nephrol* 2002; 13: 470–479
- Numata M, Nakayama M, Nimura S, Kawakami M, Lindholm B, Kawaguchi Y. Association between an increased surface area of peritoneal microvessels and a high peritoneal solute transport rate. *Perit Dial Int* 2003; 23: 116–122
- Topley N, Kaur D, Petersen MM *et al.* Biocompatibility of bicarbonate buffered peritoneal dialysis fluids: influence on mesothelial cell and neutrophil function. *Kidney Int* 1996; 49: 1447–1456
- Devuyst O. New insights in the molecular mechanisms regulating peritoneal permeability. *Nephrol Dial Transplant* 2002; 17: 548–551
- Inagi R, Miyata T, Yamamoto T *et al.* Glucose degradation product methylglyoxal enhances the production of vascular endothelial growth factor in peritoneal cells: role in the functional and morphological alterations of peritoneal membranes in peritoneal dialysis. *FEBS Lett* 1999; 463: 260–264
- Mlambo NC, Hylander B, Brauner A. Increased levels of transforming growth factor β 1 and basic fibroblast growth factor in patients on CAPD: a study during non-infected steady state and peritonitis. *Inflammation* 1999; 23: 131–139
- Beavis MJ, Williams JD, Hoppe J, Topley N. Human peritoneal fibroblast proliferation in 3-dimensional culture: modulation by cytokines, growth factors and peritoneal dialysis effluent. *Kidney Int* 1997; 51: 205–215
- Ogata S, Yorioka N, Kohno N. Glucose and prednisolone alter basic fibroblast growth factor expression in peritoneal mesothelial cells and fibroblasts. *J Am Soc Nephrol* 2001; 12: 2787–2796
- Stylianou E, Jenner LA, Davies M, Coles GA, Williams JD. Isolation, culture and characterization of human peritoneal mesothelial cells. *Kidney Int* 1990; 37: 1563–1570
- Plum J, Razeghi P, Lordnejad RM *et al.* Peritoneal dialysis fluids with a physiologic pH based on either lactate or bicarbonate buffer—effects on human mesothelial cells. *Am J Kidney Dis* 2001; 38: 867–875
- Cronauer MV, Stadlmann S, Klocker H *et al.* Basic fibroblast growth factor synthesis by human peritoneal mesothelial cells: induction by interleukin-1. *Am J Pathol* 1999; 155: 1977–1984
- De Vriese AS, Tilton RG, Stephan CC, Lameire NH. Vascular endothelial growth factor is essential for hyperglycemia-induced structural and functional alterations of the peritoneal membrane. *J Am Soc Nephrol* 2001; 12: 1734–1741
- Kang DH, Hughes J, Mazzali M, Schreiner GF, Johnson RJ. Impaired angiogenesis in the remnant kidney model: II. Vascular endothelial growth factor administration reduces renal fibrosis and stabilizes renal function. *J Am Soc Nephrol* 2001; 12: 1448–1457
- Fukasawa M, Campeau JD, Yanagihara DL, Rodgers KE, di Zerega GS. Regulation of proliferation of peritoneal tissue repair cells by peritoneal macrophages. *J Surg Res* 1990; 49: 81–87
- Feriani M, Carobi C, Greca GL, Buoncristiani U, Passlick-Deetjen J. Clinical experience with a 39 mmol/l bicarbonate-buffered peritoneal dialysis solution. *Perit Dial Int* 1997; 17: 17–21
- Feriani M, Kirchgessner J, Greca GL, Passlick-Deetjen J, and the Bicarbonate CAPD Cooperative Group. Randomized long-term evaluation of bicarbonate-buffered CAPD solution. *Kidney Int* 1998; 54: 1731–1738
- Zeier M, Schwenger V, Deppisch R *et al.* Glucose degradation products in PD fluids: do they disappear from the peritoneal cavity and enter the systemic circulation? *Kidney Int* 2003; 63: 298–305
- Liberek T, Topley N, Jorres A *et al.* Peritoneal dialysis fluid inhibition of polymorphonuclear leukocyte respiratory burst activation is related to the lowering of intracellular pH. *Nephron* 1993; 65: 260–265
- Plum J, Fleisch MC, Razeghi P *et al.* Influence of uremia on cell viability and cytokine release of human peritoneal mesothelial cells. *Kidney Blood Press Res* 2002; 25: 195–201

Received for publication: 9.12.02

Accepted in revised form: 28.6.04