Glucose and Prednisolone Alter Basic Fibroblast Growth Factor Expression in Peritoneal Mesothelial Cells and Fibroblasts

SATOSHI OGATA, NORIAKI YORIOKA, and NOBUOKI KOHNO
Second Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima, Japan.

Abstract. The mechanism of peritoneal fibrosis in patients on continuous ambulatory peritoneal dialysis is poorly understood. The production of basic fibroblast growth factor (bFGF) by human peritoneal mesothelial cells cultured in high glucose medium was investigated, and the behavior of peritoneal fibroblasts, as well as the inhibitory effect of prednisolone, was assessed. Reverse transcriptase–PCR and immunocytochemistry showed the expression of glucocorticoid receptors in mesothelial cells. The semiquantitative reverse transcriptase–PCR showed that high glucose medium (4.0%) increased bFGF mRNA by 2.5-fold relative to control medium (0.1% glucose), with 83% suppression of the increase by 1 μM prednisolone. The bFGF protein level in culture supernatant was also increased by 1.5-fold in high glucose medium, with this change showing 45% suppression by 1 μM prednisolone. These effects of prednisolone were prevented by a glucocorticoid receptor antagonist (RU486) in a concentration-dependent manner. The proliferation of peritoneal fibroblasts was increased 1.9-fold by the supernatant of mesothelial cells cultured in high glucose medium, with 85% suppression by 1 μM prednisolone and suppression to 16% below basal proliferation by an anti-bFGF neutralizing antibody (10 μg/ml), whereas proliferation showed a concentration-dependent increase on addition of an anti–transforming growth factor beta–neutralizing antibody. Recombinant bFGF (50 to 1000 pg/ml) likewise caused a concentration-dependent increase of peritoneal fibroblast proliferation and fibronectin release by these cells was also increased (at 50 to 5000 pg/ml). These results suggest the potential importance of bFGF for initiation of peritoneal fibrosis and the possible efficacy of glucocorticoids for preventing such fibrosis in patients receiving peritoneal dialysis.

Continuous ambulatory peritoneal dialysis (CAPD) has been used for two decades as a treatment for end-stage renal failure, but decreased ultrafiltration because of peritoneal fibrosis is still an important problem. Peritoneal fibrosis shows gradual progression in almost all patients receiving CAPD, irrespective of the occurrence of peritonitis. However, the mechanisms involved in the initiation and progression of such fibrosis remain to be elucidated.

In patients on CAPD, the peritoneal mesothelial layer acts as a protective barrier, and it is also involved in the transport of water and solutes, tissue remodeling after injury, and the production of many biologically active agents, including cytokines, growth factors, and adhesion molecules (1,2). Previous studies have demonstrated that a high glucose concentration and hyperosmolar peritoneal dialysate can damage mesothelial cells both in vivo and in vitro (3–6), so mesothelial injury has been suggested as the primary cause of peritoneal hypermeability, peritoneal fibrosis, or both in patients receiving CAPD.

Basic fibroblast growth factor (bFGF) is a classical cytokine, along with platelet-derived growth factor and transforming growth factor beta (TGF-β). bFGF is known to participate in the onset of fibrotic diseases (7–10). A major biologic effect of bFGF is induction of the proliferation of various cultured cells, including fibroblasts, endothelial cells, and vascular smooth muscle cells (11,12), as well as stimulation of extracellular matrix production, growth, and movement by cells of mesodermal origin such as fibroblasts (13). Although bFGF has been detected in peritoneal dialysis effluent (14,15), its actual effect on patients receiving CAPD remains to be elucidated.

Glucocorticoids, especially prednisolone, are widely used to suppress fibrosis in diseases such as interstitial pneumonia, progressive systemic sclerosis, and glomerulonephritis. However, to our knowledge, there has been no investigation into the effect of glucocorticoids on peritoneal fibrosis.

In this study, to clarify the potential mechanism of onset of peritoneal fibrosis and methods for its prevention, we investigated the following issues: (1) whether the glucocorticoid receptor is expressed by human peritoneal mesothelial cells (HPMC); (2) whether culture of HPMC in high glucose medium increases the expression of bFGF; (3) whether bFGF released by HPMC causes the proliferation of human peritoneal fibroblasts (HPFB); (4) whether prednisolone suppresses the increased production of bFGF by HPMC; and (5) whether recombinant bFGF causes the proliferation and secretion of fibronectin (an extracellular matrix component) by HPFB.

Received October 18, 2000. Accepted June 4, 2001.
Correspondence to Dr. Noriaki Yorioka, Second Department of Internal Medicine, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Phone: +81-82-255-7360; Fax: +81-82-255-7360; E-mail: nyorioka@mcai.med.hiroshima-u.ac.jp
1046-6673/1212-2787
Journal of the American Society of Nephrology
Copyright © 2001 by the American Society of Nephrology
Materials and Methods

Isolation and Culture of HPMC and Fibroblasts

HPMC and HPFB were isolated according to the methods of Stylianou et al. (16) and Beavis et al. (17), respectively. To obtain HPMC, pieces of human omentum (3 to 5 cm²) were harvested at laparotomy, washed three times with sterile phosphate-buffered saline (PBS; pH 7.3; Nissui Pharmaceutical Co., Tokyo, Japan), and then incubated with shaking in 0.125% (wt/vol) trypsin-0.01% (wt/vol) ethylenediaminetetraacetate (EDTA) solution (Life Technologies, Grand Island, NY) for 10 min at 37°C. To isolate HPFB, omental tissue that had been processed to remove mesothelial cells was incubated in trypsin-EDTA solution at 37°C with continuous rotation. Incubation was performed twice for 40 min each time, and fresh trypsin-EDTA was used during the second incubation.

Next, the tissues were removed, and 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 suspended in M199 medium supplemented with 10% fetal calf serum (FCS; vol/vol) (Mitsubishi Kasei Corp., Tokyo, Japan), 100 U/ml penicillin (Life Technologies), 100 µg/ml streptomycin (Life Technologies), and 2 mM L-glutamine (Life Technologies). After seeding into 75-cm² tissue culture flasks coated with rat type I collagen (Becton Dickinson), the cells were maintained in the same medium and incubated at 37°C in humidified incubator. The medium was replaced every 2 to 3 d.

Harvesting of omentum was approved by the medical ethics committee of Hiroshima University School of Medicine, and informed consent was obtained from each patient. All experiments were repeated three times with the cells from each donor and were performed in triplicate. HPMC from the first or second passages and HPFB from the third to fifth passages were used for the 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 (Dako, Kyoto, Japan; an epithelial and mesothelial cell marker), vimentin (Dako; a myogenic marker), and human factor VIII (Dako; an endothelial cell marker). Visualization was performed with a rhodamine-conjugated monoclonal secondary antibody (Jackson Immuno Research Laboratories, West Grove, PA) for detection of fluorescence.

Glucocorticoid Receptor mRNA and Protein Expression

Reverse transcriptase-PCR (RT-PCR) and immunocytochemistry were performed to detect glucocorticoid receptor mRNA and protein in HPMC. HPMC and human umbilical vein endothelial cells (HUVEC) obtained from the American Type Culture Collection (Rockville, MD) as a positive control (18) were cultured in 60-mm dishes (both at 1 × 10⁶ cells/dish) coated with rat type I collagen (Becton Dickinson). After each culture was confirmed to be subconfluent by microscopy, the cells were washed twice with PBS, and the medium was replaced with a test medium. After 6, 12, 24, or 48 h, the cells were again washed twice with PBS, total RNA was extracted according to the method mentioned above, and RT-PCR was performed with denaturation for 1 min at 95°C, annealing for 1 min at 56°C, and extension at 72°C for 2 min. After 40 cycles, the final product was extended for 3 min at 72°C. A Program Temp Control System PC-800 (Astec, Fukuoka, Japan) was used. PCR products were separated by 2% agarose gel electrophoresis and were visualized by ethidium bromide staining. Non–reverse-transcribed RNA was used as a template for the negative control.

By use of the standard avidin-biotin complex method, immunocytochemistry was performed on cytospin samples of HPMC (1 × 10⁶ cells/slide), and on HUVEC as a positive control. Briefly, the cells were air dried, fixed in cold acetone at −10°C for 10 min, and rehydrated in PBS, after which endogenous peroxidase was blocked by incubation with 0.3% hydrogen peroxide. Next, the cells were incubated with diluted normal blocking serum (Vector Labs, Burlingame, CA) for 20 min at room temperature to avoid nonspecific staining. This was followed by overnight incubation at 4°C with the primary antibody, a rabbit polyclonal anti-human glucocorticoid receptor antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) diluted to 1:100 in PBS. Incubation without the primary antibody was also performed as the negative control. After three washes in PBS, the cells were sequentially incubated with a biotinylated goat anti-rabbit antibody (Vector Labs), the ABC-Elite reagent (Vector Labs), and finally with 3,3’-diaminobenzidine. Then the cells were dehydrated in an alcohol series and covered with a cover slip.

Culture Media and Cell Viability

Ten different culture media were used. Standard M199 medium containing 10% FCS (vol/vol), 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mM L-glutamine was used as the control medium with a glucose concentration of 0.1% (5.6 mM). This medium was supplemented with d-glucose (Kanto Chemical Co., Inc., Tokyo, Japan) at a concentration of 1.5% (84 mM), or 4.0% (222 mM), or with prednisolone (Shionogi Co. Ltd., Osaka, Japan) at various concentrations (0.01, 0.1, and 1 µM). In some experiments, a glucocorticoid receptor antagonist (RU486; mifepristone; Sigma Chemical Co., St. Louis, MO) was added at 0.1, 1, 10, and 100 µM to medium containing 4.0% glucose and 1 µM prednisolone. All media were sterilized by filtration (0.22 µm, Millex-GV, Millipore, Bedford, MA) and then stored at 4°C for use within 3 d.

We examined the viability of HPMC (1 × 10⁵ cells/well) cultured in these 10 media in 96-well plates coated with rat type I collagen (Becton Dickinson) by the [%H]-thymidine incorporation assay at the times mentioned below.

Semi-Quantification of bFGF mRNA in HPMC by RT-PCR

After HPMC (1 × 10⁶ cells/dish) cultured in 60-mm dishes were confirmed to be subconfluent by microscopy, the cells were washed twice with PBS, and the medium was replaced with a test medium. After 6, 12, 24, or 48 h, the cells were again washed twice with PBS, total RNA was extracted by the method mentioned above, and RT-PCR was performed to semiquantitatively measure bFGF mRNA by the method of Maeda et al. (21). In a preliminary study, the PCR was performed with 1 µl of cDNA; various cycle patterns were used to determine the number of cycles at which gene amplification was exponential for each primer, and 25 cycles were adopted for the experiment. The oligonucleotide primer sets were as follows: bFGF (22): sense 5′-ATGGCAGCCGGGAGCATCCTG-3′, antisense 5′-GCAGCACCTGATGTGAGGTG-3′. These primers yielded a 536-bp product. PCR was performed with denaturation for 1 min at 95°C, annealing for 1 min at 56°C, and extension at 72°C for 2 min. After 40 cycles, the final product was extended for 3 min at 72°C. A Program Temp Control System PC-800 (Astec, Fukuoka, Japan) was used. PCR products were separated by 2% agarose gel electrophoresis and were visualized by ethidium bromide staining. Non–reverse-transcribed RNA was used as a template for the negative control.

By use of the standard avidin-biotin complex method, immunocytochemistry was performed on cytospin samples of HPMC (1 × 10⁶ cells/slide), and on HUVEC as a positive control. Briefly, the cells were air dried, fixed in cold acetone at −10°C for 10 min, and rehydrated in PBS, after which endogenous peroxidase was blocked by incubation with 0.3% hydrogen peroxide. Next, the cells were incubated with diluted normal blocking serum (Vector Labs, Burlingame, CA) for 20 min at room temperature to avoid nonspecific staining. This was followed by overnight incubation at 4°C with the primary antibody, a rabbit polyclonal anti-human glucocorticoid receptor antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) diluted to 1:100 in PBS. Incubation without the primary antibody was also performed as the negative control. After three washes in PBS, the cells were sequentially incubated with a biotinylated goat anti-rabbit antibody (Vector Labs), the ABC-Elite reagent (Vector Labs), and finally with 3,3’-diaminobenzidine. Then the cells were dehydrated in an alcohol series and covered with a cover slip.
product). The primer sets used in this experiment were designed so that at least one intron was located in the corresponding genomic sequence to detect possible amplification of any contaminating genomic DNA. PCR involved 25 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 56°C, and extension at 72°C for 2 min, after which the final product was extended for 3 min at 72°C. PCR products were separated by 2% agarose gel electrophoresis and were visualized by ethidium bromide staining. The density of the PCR products was measured with a charge-coupled device imaging system (Densitograph AE-6900MF; Atto, Tokyo, Japan). The bFGF band was normalized for the β-actin band, and each measurement was performed twice.

Measurement of bFGF Secretion
Supernatants were collected after mesothelial cells (1 × 10^5/well) had been cultured for 6, 12, 24, or 48 h with various media (1.0 ml/well) in 24-well dishes coated with rat type I collagen (Becton Dickinson) and the bFGF level in the culture supernatants was measured with a sandwich enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN).

Effect of a Glucocorticoid Receptor Antagonist (RU486)
Various concentrations of RU486 were added to medium containing 4.0% glucose and 1 μM prednisolone. Then bFGF mRNA expression by HPMC was assessed after incubation for 6 h, and the bFGF protein concentration in the HPMC supernatant was measured after incubation for 24 h by the methods mentioned above.

Proliferation of Fibroblasts Exposed to HPMC Supernatant
Supernatants were collected after mesothelial cells (4 × 10^6 cells/flask) had been cultured for 24 h with various media (10 ml/75 cm²) in T-75 flasks coated with rat type I collagen (Becton Dickinson). Proliferation of HPFB was measured by the [3H]-thymidine incorporation assay. The cells (5 × 10^3/well) were plated into 96-well plates (Becton Dickinson) and were incubated in M199 medium without FCS for 24 h to induce quiescence. Then the cells were incubated for 72 h in HPMC supernatant (200 μl/well) with or without an anti-bFGF neutralizing antibody (0.1, 1, or 10 μg/ml; Wako, Osaka, Japan), an anti–TGF-β1 neutralizing antibody (0.5, 1, 5, or 10 μg/ml, R&D Systems), or mouse IgG (10 μg/ml, Sigma), and were pulsed with 1 μCi of [3H]-thymidine (Amersham Pharmacia Biotech, Buckinghamshire, England) for the last 6 h. In a preliminary study, we evaluated the proliferation of HPFB after incubation for 24, 48, and 72 h. Because we found significant changes at 72 h, but not 24 or 48 h, we adopted 72 h as the time of assessment. Subsequently, the cells were washed twice in ice-cold PBS, precipitated twice with 10% trichloroacetic acid, resuspended in 0.5 M NaOH, and neutralized with 1 M HCl. Then the radioactivity was counted with a liquid scintillation counter (LS5100; Aloka, Tokyo, Japan).

The effect of HPMC supernatant diluted with PBS (x1, x1/2, x1/10, x1/20, and x1/100) was also examined via the [3H]-thymidine incorporation assay. Test media (200 μl/well) were added to 5 × 10^4 quiescent HPFB in 96-well plates. Incubation was done for 72 h, and the cells were pulsed with 1 μCi of [3H]-thymidine (Amersham Pharmacia Biotech) for the last 6 h.

Effect of Recombinant bFGF on Peritoneal Fibroblasts
Proliferation of HPFB in response to recombinant bFGF was assessed via the [3H]-thymidine incorporation assay. Test media (M199 medium with 0 to 1000 pg/ml of recombinant human bFGF: Pepro Tech Inc., Rocky Hill, NJ) were added at 200 μl/well to quiescent HPFB (5 × 10^4/well) in 96-well plates. Incubation was done for 72 h, and the cells were pulsed with 1 μCi of [3H]-thymidine (Amersham Pharmacia Biotech) for the last 6 h.

Production of fibronectin by HPFB was evaluated by measuring fibronectin levels in the culture supernatants. HPMC (3 × 10^4/well) were plated in 24-well plates (Becton Dickinson) after incubation in M199 medium without FCS for 24 h to induce quiescence. Supernatants were collected after culture for 24 h in M199 medium (1.0 ml/well) containing recombinant human bFGF (0–5000 pg/ml) without FCS, and the level of fibronectin protein was measured in the supernatant with an enzyme-linked immunosorbent assay kit (Biochemical Technologies, Inc., Stoughton, MA).

Statistical Analyses
Statistical analyses were performed by the unpaired or paired t test, as appropriate. Results are expressed as the mean ± SEM; P < 0.05 was considered statistically significant. The suppression rate (percentage decrease of a response) for prednisolone or anti-bFGF neutralizing antibody was calculated as follows: [response to 4.0% glucose medium (or its supernatant) – response to the test medium]/[response to 4.0% glucose medium (or its supernatant) – response to 0.1% glucose control medium (or its supernatant)] × 100.

Results
Identification of HPMC and HPFB
The cultured cells were identified as mesothelial cells by their typical cobblestone appearance at confluence (Figure 1A), as well as by positive staining for cytokeratin and vimentin plus negative staining for factor VIII on immunocytochemistry. Fibroblasts were identified by their typical bipolar and multipolar morphology (Figure 1B), along with positive staining for vimentin and negative staining for cytokeratin and factor VIII. Cultures were excluded when the other type of cell was found as a contaminant.

Glucocorticoid Receptor Expression by HPMC
RT-PCR was used to detect the expression of glucocorticoid receptor mRNA by cultured HPMC (Figure 2). Immunocytochemistry revealed strong expression of glucocorticoid receptor protein in the nucleus and weak expression in the cytoplasm of all HPMC (Figure 3).

HPMC Viability
The viability of HPMC showed a significant decrease in a time-dependent manner (0.1% glucose: 4.70 ± 0.43 × 10^4 cpm at 6 h versus 1.86 ± 0.55 × 10^4 cpm at 48 h [P < 0.01], and 4.0% glucose: 4.33 ± 0.49 × 10^4 cpm at 6 h versus 1.02 ± 0.82 × 10^4 cpm at 48 h [P < 0.01]), but there were no significant differences among all of the test media with respect to bFGF mRNA expression and protein secretion at any specific time of assessment (Figure 4).

bFGF mRNA Expression by HPMC
The maximum difference between control cultures (0.1% glucose) and 4.0% glucose medium was seen at 6 h in the experiments on bFGF mRNA expression (Figure 5). At 6 h,
confluent HPMC showed bFGF mRNA expression, and the mean ± SEM of the bFGF mRNA to β-actin mRNA ratio was 0.55 ± 0.05 in control cultures. Expression of bFGF mRNA showed a glucose concentration-dependent increase, with a 2.5-fold increase (1.37 ± 0.17) at 4.0% glucose relative to control medium (P < 0.01). There was 83% suppression of this increase by incubation with 1 μM prednisolone (0.69 ± 0.16) (P < 0.05) (Figure 6).

**bFGF Secretion by HPMC**

The maximum difference between control cultures (0.1% glucose) and 4.0% glucose medium was found at 24 h in the experiments on bFGF protein secretion (Figure 7). Confluent HPMC secreted bFGF at 24 h, and the mean ± SEM bFGF level in the culture supernatant was 412 ± 35 pg/ml per 10⁵ cells in control cultures. There was a glucose concentration-dependent increase of bFGF secretion, with a 1.5-fold increase (631 ± 29 pg/ml per 10⁵ cells) in 4.0% glucose medium relative to control cultures (P < 0.01). This increase showed 45% suppression by 1 μM prednisolone (533 ± 25 pg/ml per 10⁵ cells) (P < 0.05) (Figure 8).

**Effect of a Glucocorticoid Receptor Antagonist (RU486)**

The above-mentioned effect of prednisolone on bFGF mRNA expression was prevented by RU486 in a concentration-dependent manner (100 μM: 1.42 ± 0.14, P < 0.05; Figure 9A). Likewise, RU486 showed concentration-dependent reversal of the effect on prednisolone on protein secretion (100 μM: 587 ± 11 pg/ml per 10⁵ cells, P < 0.05; Figure 9B).

**Fibroblast Proliferation in Response to HPMC Supernatant**

Proliferation of HPFB was increased in a glucose-concentration-dependent manner by HPMC supernatant, and a 1.9-fold increase relative to control cultures was seen after exposure to supernatant from HPMC cultured with 4.0% glucose (14.8 ± 1.1 versus 7.90 ± 0.53 × 10⁵ cpm; P < 0.05). This increased proliferative response showed 85% suppression after the addition of 1 μM prednisolone (8.92 ± 1.00 × 10⁵ cpm; P < 0.05; Figure 10). Proliferation was also suppressed in a concentration-dependent manner by addition of an anti-bFGF neutralizing antibody (suppression to 16% below basal proliferation at 10 μg/ml, 0.68 ± 0.06 × 10³ cpm) to supernatant from HPMC (P < 0.01), but was not suppressed by addition of mouse IgG at 10 μg/ml (1.40 ± 0.12 × 10³ cpm; Figure 11). Addition of an anti–TGF-β1 neutralizing antibody increased HPFB proliferation in a concentration-dependent fashion (Figure 11). Dilution of the HPMC supernatant reduced fibroblast proliferation in relation to the extent of dilution (Figure 12).

**Effect of Recombinant bFGF on Peritoneal Fibroblasts**

There was a concentration-dependent increase of HPFB proliferation after culture for 72 h with recombinant human bFGF at concentrations from 50 to 1000 pg/ml (control: 0.36 ±
Discussion

This study demonstrated the following points for the first time: (1) HPMC express the glucocorticoid receptor; (2) culture of HPMC in high glucose medium increases bFGF expression; (3) bFGF secreted by HPMC can stimulate the proliferation of peritoneal fibroblasts; and (4) prednisolone suppresses bFGF secretion by HPMC, acting via the glucocorticoid receptor. We also showed that (5) recombinant bFGF can promote the proliferation and the secretion of fibronectin by HPFB.

Several reports have indicated the importance of TGF-β in the mechanism of peritoneal fibrosis (24–26), but recent studies have shown that FGF enhances the proliferation of cultured HPFB and has an effect far stronger than that of TGF-β. Beavis et al. (17) and Fukuwada et al. (27) 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 of bFGF in the mechanism of peritoneal fibrosis, and this study obtained findings supporting these reports.

It was previously reported that recombinant bFGF, peritoneal dialysis effluent, and HPMC-conditioned medium all have a mitogenic effect on peritoneal fibroblasts, with the proliferative effect of the effluent being reduced by coincubation with an anti-bFGF antibody (17). However, the peritoneal source of...
bFGF was not clarified, and neither was the relationship between bFGF and the glucose concentration. In the study presented here, we showed that glucose caused a concentration-dependent increase in the production of bFGF by HPMC, indicating that these cells are one of the peritoneal sources of this factor.

This study assessed the effect on HPMC of incubation with 1.5% (84 mM) glucose and 4.0% (222 mM) glucose, which are the glucose concentrations found in commercial peritoneal dialysates. In the presence of high glucose, we confirmed that there was a concentration-dependent increase of bFGF mRNA expression and bFGF protein secretion by HPMC. Thus, not only the release of bFGF but also its production was promoted by high glucose. Because of the lack of a sequence characteristic of the secreted protein, the mode of bFGF secretion is unclear, but it was previously shown that lethal cell injury releases intracellular bFGF (28) and that sublethal injury
causes the liberation of active bFGF from its cytosolic storage sites by plasma membrane disruption (29). Accordingly, bFGF may be involved in the regulation of repair and remodeling after injury to the peritoneum.

Previous studies have shown that the viability of HPMC decreases in medium with a high glucose concentration (4,24,26). This study showed a decrease of viability due to high glucose medium, but the change was not significant. On the other hand, the viability of HPMC showed a significant decrease in a time-dependent manner, especially after 24 to 48 h. This might be a reason why the secretion of bFGF decreased markedly after 48 h.

Hyperplasia of fibroblasts is observed in various fibrotic diseases, such as bone marrow fibrosis, scleroderma, and pulmonary fibrosis (30–32). The ultrastructural changes observed by peritoneal biopsy in patients on CAPD include submesothelial fibrosis (33–35) resulting from fibroblast hyperplasia and increased deposition of extracellular matrix (ECM) (36,37). Factors that cause the proliferation of fibroblasts and increase ECM are important in fibrotic diseases, but the mechanism underlying the accumulation of ECM in the mesothelium of patients receiving CAPD remains unknown. It was previously shown that exposure to high glucose levels upregulated the expression of fibronectin mRNA and protein by HPMC (38).

It has been suggested that altered degradation of the ECM may be an important factor in its accumulation, as well as either increased ECM synthesis or increased incorporation into the matrix (39). Clinically, it has been found that peritoneal fibrosis develops slowly in patients receiving CAPD after several years of treatment (33–35), suggesting that an imbalance between ECM synthesis and degradation may be important in its progression. In this study, we showed that bFGF is mitogenic for HPFB and increases fibronectin secretion by these cells. Thus, bFGF released from damaged mesothelial cells may have an important role in promoting fibroblast proliferation and accumulation of ECM in the process of peritoneal fibrosis.

In an attempt to prevent peritoneal fibrosis, various improve-
ments have been made to CAPD fluid (which lacks a physiologic basis because of a high glucose content, low pH, lactate, and hyperosmolality) by the use of icodextrin and sodium bicarbonate; the prevention of peritonitis has been facilitated by the adoption of a twin-bag system and connecting devices sterilized by ultraviolet light. However, there have been few reports of drug therapy that use antifibrotic agents.

Expression of the glucocorticoid receptor has already been investigated in monocytes, mesangial cells, macrophages, and HUVEC (18,40–42), but not in HPMC. In this study, the receptor was strongly positive in the nucleus and mildly positive in the cytoplasm of HPMC. Regarding the localization of the glucocorticoid receptor, Yan et al. (41) and Picard et al. (43) showed that this receptor could be detected in both the nucleus and the cytoplasm and that the nuclear receptor was increased by dexamethasone or FCS. Accordingly, our use of FCS in the culture medium may have increased nuclear expression of the receptor.

Glucocorticoids bind to their cytosolic receptor, which is normally associated with two molecules of a 90-kD heat shock protein (HSP90). The glucocorticoid/glucocorticoid receptor complex then translocates to the nucleus and binds to the glucocorticoid response element in the promoter sequences of various target genes, resulting in an increase or a decrease of transcription (44). RU486 stabilizes the association of steroid receptors with HSP90, which prevents the translocation of the glucocorticoid receptor to the nucleus and thereby blocks transcription of genes containing a glucocorticoid-responsive element (45). Our demonstration that RU486 reverses the effects of prednisolone on the expression of bFGF by HPMC shows that the actions of prednisolone are mediated through the glucocorticoid receptor.

There have been several reports about the relationship between bFGF and glucocorticoids, but the effect of glucocorticoids on bFGF expression remains uncertain. Peifley et al. (46) showed that heparin-binding epidermal growth factor–like growth factor induced bFGF mRNA expression in aortic vascular smooth muscle cells and that this change could be inhibited by glucocorticoid therapy. In PC12 cells, however, glucocorticoids stimulated the expression of bFGF, and no glucocorticoid effect on bFGF expression was found in the adrenal cortex, heart, skeletal muscle, and kidney (47). The study presented here suggested that glucocorticoid therapy
could be of benefit to patients receiving CAPD under some circumstances, but its use would need to be monitored carefully with respect to side effects including the risk of peritonitis.

In contrast to the potential involvement of bFGF released by HPMC in the initiation of proliferation and fibronectin production by HPFB during the early phase of peritoneal fibrosis, our data do not support a role for bFGF in the later phase of fibrosis, because after several years of CAPD, there are changes in HPMC morphology and the cells start to show detachment from the basement membrane (6,33,35).

In conclusion, our results suggest the importance of bFGF in the mechanism of onset of peritoneal fibrosis, and they also suggest the potential efficacy of glucocorticoids for preventing peritoneal fibrosis in patients receiving CAPD.

Acknowledgments

We thank Dr. Toshimasa Asahara, Dr. Seiji Marubayashi, Dr. Hiroshi Yahata (Second Department of Surgery, Hiroshima University School of Medicine), Dr. Shirou Nakai, and Dr. Toru Ogata (Department of Surgery, Hiroshima Memorial Hospital) for their cooperation in collecting samples during abdominal surgery.

References

27. Fukasawa M, Campeau JD, Yanagihara DL, Rodgers KE, diZerega GS: Regulation of proliferation of peritoneal tissue...


