Peritoneal Dialysis International Peritoneal Dialysis International Peritoneal Dialysis International

Proceedings of the Fourth European PD Meeting
Madrid, Spain, 15-18 April 2000
Peritoneal Dialysis International, Vol. 21, pp. 219-224
0896-8608/00 $3.00 + .00
Copyright © 2001 International Society for Peritoneal Dialysis
Printed in Canada. All rights reserved.

DOES ENALAPRIL PREVENT PERITONEAL FIBROSIS INDUCED BY HYPERTONIC (3.86%) PERITONEAL DIALYSIS SOLUTION?

Soner Duman,[1] Ali Ihsan Günal,[1] Sait Sen,[2] Gülay Asçi,[1] Mehmet Özkahya,[1] Ender Terzioglu,[3] Fehmi Akçiçek,[1] and Gürhun Atabay[1]

Departments of Nephrology,[1] Pathology,[2] and Immunology,[3]
Ege University Medical School, Izmir, Turkey

♦ ***Objective:*** **Peritoneal fibrosis (PF) is one of the most serious causes of failure in continuous ambulatory peritoneal dialysis (PD). Although the underlying mechanism responsible for the genesis of PF is still unknown, transforming growth factor β ($TGFβ_1$) has been shown to be associated with PF. Angiotensin converting enzyme inhibitors have been shown to prevent the stimulating effect of growth factors. The aim of the present study was to investigate the effect of enalapril on peritoneal function and morphology in a rat model of experimental PF.**

♦ ***Methods:*** **Twenty-one albino Wistar rats were divided into three groups: (1) the control group (C) received 10 mL isotonic saline intraperitoneally (IP), (2) the dextrose (Dx) group 10 mL 3.86% dextrose PD solution IP, and (3) the enalapril-treated group (ENA) 10 cc 3.86% dextrose PD solution IP plus 100 mg/L enalapril in drinking water. After 4 weeks, a 1-hour peritoneal equilibration test was performed with 20 mL 2.27% dextrose PD solution. Dialysate-to-plasma urea ratio (D/P urea), glucose reabsorption (D_1/D_0 glucose), ultrafiltration (UF) volume, and levels of dialysate protein, $TGFβ_1$, and cancer antigen 125 (CA125) were determined. The parietal peritoneum was evaluated histologically by light microscopy.**

♦ ***Results:*** **Administration of enalapril resulted in preserved UF (–0.2 ± 0.7 mL vs 1.7 ± 0.3 mL, $p < 0.05$), protein loss (2.3 ± 0.5 g/L vs 1.6 ± 0.2 g/L, $p > 0.05$), and peritoneal thickness (77 ± 7 μ vs 38 ± 5 μ, $p < 0.001$). D/P urea increased significantly in the Dx group ($p < 0.05$). Both higher levels of $TGFβ_1$ (undetectable vs 298 ± 43 pg/mL, $p < 0.001$) and lower levels of CA125 in dialysate effluent (0.94 ± 0.5 U/L vs 0.11 ± 0.1 U/L, $p > 0.05$) were determined in the Dx group.**

♦ ***Conclusion:*** **These findings show that peritoneal morphology and function tests were dramatically deranged in the Dx group. The same properties were partially preserved in the ENA group. The production of $TGFβ_1$ was significantly reduced but peritoneal thickness was not completely inhibited. In conclusion, by inhibiting the production of $TGFβ_1$, enalapril can preserve peritoneal histology, peritoneal function, and remodeling of mesothelial cells.**

KEY WORDS: Peritoneal fibrosis; ACE-I; $TGFβ_1$; CA125; enalapril.

Ultrafiltration failure (UFF) is the most common cause of functional transport abnormality and dropout from continuous ambulatory peritoneal dialysis (CAPD) in long-term peritoneal dialysis (PD). The prevalence of UFF increases from 3% during the first year on CAPD to 31% at 6 years (1–3). During CAPD, various morphological changes take place in the peritoneum, including mesothelial denudation, interstitial fibrosis, neovascularization, and vascular alterations (replication of basement membrane, fibrosis and hyalinization of the vascular wall) (4,5). Among the suggested causes of these histological and functional alterations are recurrent peritonitis, plasticizers, advanced glycosylation end-products, and the nonphysiological nature of the PD solutions, in particular high glucose content, hypertonicity, lactate, and low pH (5–7). Although the mechanisms underlying these alterations are not completely understood, growth factors and cytokines secreted by mesothelial cells (MCs) and macrophages have been shown to be associated with peritoneal fibrosis (8). Dobbie suggested the MCs' injury resulted from the chronic irritative effect of the dialysis solutions, and that severe or prolonged peritonitis is the

Correspondence to: S. Duman, Ege Üniversitesi Tip Fakültesi, Nefroloji Bilim Dali, Bornova 35100, Izmir, Turkey.
dumans@med.ege.edu.tr
Received 15 August 2000; accepted 21 December 2000.

Downloaded from http://www.pdiconnect.com/ by guest on February 23, 2013

initiating event that leads to peritoneal fibrosis (9). Mesothelial cells can cause peritoneal fibrosis by secreting extracellular matrix macromolecules (ECM) consisting of collagen, fibronectin, laminin, proteoglycans, and cytokines, including transforming growth factor beta 1 ($TGF\beta_1$) and interleukin-1 (IL-1).

$TGF\beta_1$, a key mediator of ECM accumulation in fibrotic diseases, has several properties that cause fibrosis. These include (1) increased synthesis of matrix proteins such as collagens, fibronectin, and proteoglycans; (2) decreased degradation of matrix proteins by suppression of protease expression and increased production of protease inhibitors, such as tissue inhibitor of matrix metalloproteinase and plasminogen-activator-inhibitor type 1; and (3) increased synthesis of integrins (12). Reduction of $TGF\beta_1$ overexpression decreases pathological matrix accumulation. In rats with glomerulonephritis, it has been shown that treatment with $TGF\beta_1$ neutralizing antibodies or the natural $TGF\beta_1$ antagonist, decorin, decreases glomerular matrix accumulation (12,13).

Angiotensin (ANG) II plays a role in stimulating macrophages and fibroblast-like cells to secrete $TGF\beta_1$. A perivascular/interstitial fibrosis, for instance, accompanies chronic elevation in either circulating ANG II or aldosterone (10) and, in case of ANG II, occurs in response to abnormal vascular permeability and escape of macromolecules (11).

It has been shown that activated macrophages isolated from a peritoneal exudate that forms after instillation of mineral oil into the peritoneal space express ANG II receptors (14). In the literature, there are many experimental studies showing the beneficial effect of angiotensin-converting enzyme inhibitors (ACE-I). For instance, radiation-induced glomerular sclerosis is prevented by enalapril (15). Interstitial scarring, induced by cyclosporine, is prevented by enalapril or losartan begun at the time of administration of this nephrotoxin (16). Captopril treatment causes a decrease in renal growth and urinary albumin excretion in experimental diabetic nephropathy (17).

In view of the above findings, we performed an experimental study to investigate the effect of enalapril treatment on peritoneal alterations induced by hypertonic PD solutions in rats.

MATERIALS AND METHODS

The study was performed in nonuremic male albino Wistar rats weighing 200 – 220 g. The rats were housed in polycarbonate cages and were fed a standard laboratory diet and allowed free access to water. The temperature was maintained at 30°C with a 12-hour light/dark cycle. The Animal Ethics Committee of the Ege University Medical School approved the study design. The animals were divided into three groups: the control group (7 rats) received 10 mL isotonic saline intraperitoneally (IP); the dextrose group (Dx, 7 rats) received 10 mL 3.86% dextrose PD solution IP; the enalapril-treated group (ENA, 7 rats) received 10 mL 3.86% dextrose PD solution IP and 100 mg/L enalapril in their drinking water. Each rat consumed approximately 10 – 15 mL drinking water (including 1.0 – 1.5 mg enalapril) daily. The enalapril dosage was adjusted according to Peter *et al.*'s study because they showed that the maximum $TGF\beta_1$-inhibiting dose of enalapril was 100 mg/L in drinking water (11). All injections were performed once daily IP for 4 weeks.

After the 4 weeks, a 1-hour peritoneal equilibration test (PET) was performed: 20 mL 2.27% dextrose PD solution (37°C) was slowly injected, using a 22-gauge needle, into the peritoneal cavity of the rats. After the injection the rats were allowed to become ambulatory and had free access to water. At the end of 1 hour, the animals were anesthetized with ketamine (60 mg/kg body weight). A shortened PD catheter was inserted into the peritoneal cavity through a midline incision without any dialysate leakage from the peritoneal cavity. Immediately thereafter, a sample of dialysate was collected and a blood sample was collected by direct cardiac puncture. The peritoneal cavity was then opened and checked for residual fluid, and a sample of parietal

Downloaded from http://www.pdiconnect.com/ by guest on February 23, 2013

Peritoneal Dialysis International

Peritoneal Dialysis International

peritoneum at a place far from the injection site was taken. Peritonitis developed in one rat in the Dx group and two in the ENA group; both were withdrawn from the study.

In the histological examination, the peritoneal membrane sections were fixed in 10% buffered formalin solution at room temperature and processed routinely for light microscopy. Paraffin sections were cut (5 μ) and stained by hematoxylin & eosin and Masson trichrome. Selected sections were also stained by immunoperoxidase methods for collagen III and collagen IV (Dako; Glostrup, Denmark).

LIGHT MICROSCOPY

The sections were examined by light microscopy by the same pathologist, with no indication of group. Number and reactivity of MCs; change in basal lamina; presence of inflammation, submesothelial edema, fibroblastic activity and fibrosis, and vascularization; and peritoneal thickness were evaluated. Inflammation, fibroblastic activity, and neovascularization were scored semiquantitatively by counting mononuclear cells, fibroblasts, and capillaries per high power field at ×400 magnification (score 0 – 3). Fibrosis also was scored as an evaluation of edema and collagen density.

Mesothelial cells were counted as the mean of five different areas (cells/high power field at ×400 magnification) and classified as normal, decreased, or increased. Mesothelial cells were classified as either normal (flat cells) or reactive (cubic transformation of flat cells). Basal lamina was evaluated as normal (size of red blood cell), thick, or thin. Fibrosis was evaluated as absent, early (edema and a few lacy collagen), middle (lacy and mature collagen), or late (mature collagen fibrils).

MORPHOMETRIC ANALYSIS

Peritoneal interstitial thickness was also measured from the inner surface of the muscle to the mesothelium. Peritoneal interstitial thickness values were calculated as the mean of three different areas measured by two independent observers.

Blood and dialysate urea were measured using an enzymatic kinetic method (Randox Laboratories, San Francisco, CA, U.S.A.). Glucose concentrations were determined using a glucose oxidase method. Dialysate protein was measured using a turbidimetric assay. Net UF was measured as the difference between the instilled and drained dialysate. D/P urea was calculated. D_1/D_0 was determined by the ratio of glucose concentration in the drained dialysate to the unused fluid.

Dialysate $TGF\beta_1$ level was measured using a commercially available enzyme-linked immunosorbent assay kit (Promega, Madison, WI, U.S.A.) following manufacturer's instructions. $TGF\beta_1$ antibody cross-reactivity with other $TGF\beta$ isoforms was as follows: $TGF\beta_{1.2}$ heterodimer, 11.5%; $TGF\beta_2$, 1.6%; and $TGF\beta_3$, 0.7%. Dialysate CA125 level was determined using a microparticle enzyme immunoassay in combination with a commercially available monoclonal antibody (Abbott Laboratories; Chicago, IL, U.S.A.) on an IMx auto analyzer (Abbott Laboratories).

All data are reported as mean ± SEM. Data were analyzed using unpaired t-test for the results given in Table 1 and using the Mann–Whitney U test for the results given in Table 3. A p value of less than 0.05 was considered significant.

RESULTS

The results of the study are summarized in Table 1. Our results indicate that administration of 3.86% dextrose PD solution into the peritoneal cavity for 4 weeks alters peritoneal structure and affects function. Significant alterations in peritoneal function, such as decreased D_1/D_0 glucose ratio (0.39 ± 0.08 vs 0.56 ± 0.05, $p < 0.05$), increased D/P urea (0.72 ± 0.1 vs 0.48 ± 0.2, $p < 0.05$), increased protein loss (2.3 ± 1.2 g/L vs 1.5 ± 0.4 g/L, $p > 0.05$), and loss of UF capacity (–0.2 ± 1.9 mL vs 5.8 ± 0.9 mL, $p < 0.01$) were determined in the rats administered only hypertonic PD solution compared to control. A higher D_1/D_0 glucose ratio (0.42 ± 0.07 vs 0.39 ± 0.08,

Downloaded from http://www.pdiconnect.com/ by guest on February 23, 2013

TABLE 1
Functional Findings of Peritoneum in Rats Administered Different Treatments

	Control (C)	Dextrose (D)	Dextrose + Enalapril (E)	*p* Value
Ultrafiltration (mL)	5.8±0.3	−0.2±0.7	1.7±0.3	<0.001 C vs D, C vs E; <0.05 D vs E
D/P urea	0.48±0.08	0.72±0.04	0.57±0.04	<0.05 C vs D
D_1/D_0	0.56±0.02	0.39±0.04	0.42±0.02	<0.01 C vs D, C vs E
Protein loss (g/L)	1.5±0.2	2.3±0.5	1.6±0.2	>0.05
TGFβ_1 (pg/mL)	Undetectable	298±43	Undetectable	0.001 C vs D, D vs E
CA125 (U/mL)	0.35±0.2	0.11±0.1	0.94±0.5	>0.05

D/P = dialysate-to-plasma ratio; D_1/D_0 = glucose reabsorption; TGF = transforming growth factor; CA125 = cancer antigen 125.

TABLE 2
Semiquantitative Data for Mesothelial Cells and Submesothelial Area

Mesothelium	Control	Dextrose	Dextrose+ enalapril
Cell morphology			
Normal	7	1	4
Reactive	—	4	2
Cell number			
Normal	4	—	3
Decreased	3	3	3
Increased	—	2	—
Basal lamina			
Normal	4	1	—
Thick	3	3	4
Thin	—	1	2
Fibrosis			
Absent	4	—	—
Early	3	3	3
Middle	—	2	3
Late	—	—	—

$p > 0.05$), a lower D/P urea (0.57 ± 0.1 vs 0.72 ± 0.1, $p < 0.05$), a lower protein loss (1.6 ± 0.5 g/L vs 2.3 ± 1.2 g/L, $p > 0.05$), and a higher UF capacity (1.7 ± 0.8 mL vs −0.2 ± 1.9 mL, $p < 0.05$) were determined in the ENA group compared to the Dx group (Table 1). The peritoneal membrane in the Dx group was thicker than that of the control and ENA groups (77 ± 17 μ, 38 ± 12 μ, 4.7 ± 0.4 μ, respectively; $p < 0.01$) (Table 2).

TABLE 3
Morphological Results for Peritoneum in Rats Under Different Treatments

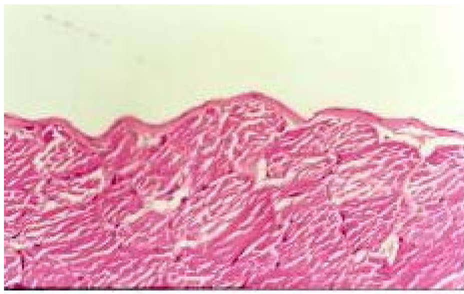
	Control (C)	Dextrose (D)	Dextrose + enalapril (E)	*p* Values
Thickness (μ)	4.7±0.40	77.8±7.20	38±5.04	C vs D[a]; C vs E[a]; D vs E[a]
Inflammation	0.00±0.00	1.20±0.20	0.33±0.21	C vs D[b]; C vs E[c]; D vs E[c]
Fibroblasts	0.43±0.20	1.00±0.32	0.67±0.21	C vs D[c]; C vs E[c]; D vs E[c]
Neovascularization	0.14±0.38	1.40±0.24	1.00±0.00	C vs D[c]; C vs E[b]; D vs E[c]
Mesothelial cells	11.14±1.86	14.44±4.40	12.44±1.56	C vs D[c]; C vs E[c]; D vs E[c]

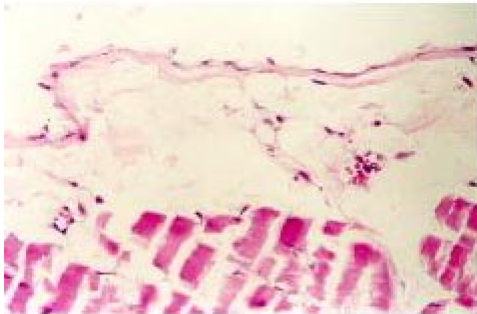
[a] $p < 0.01$.
[b] $p < 0.05$.
[c] $p > 0.05$.

Downloaded from http://www.pdiconnect.com/ by guest on February 23, 2013

Peritoneal Dialysis International Peritoneal Dialysis International Peritoneal Dialysis International

Peritoneal Dialysis International Peritoneal Dialysis International Peritoneal Dialysis International





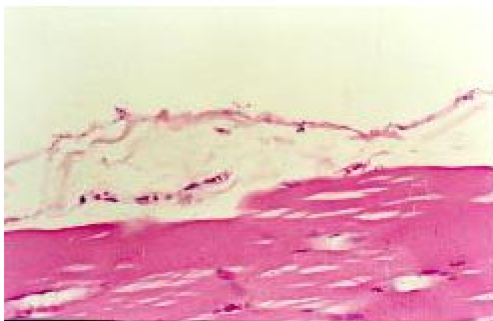


Figure 1 — Morphology of the peritoneum in control (upper), hypertonic dextrose alone (middle), and hypertonic dextrose plus enalapril groups (lower). (Hematoxylin & eosin, ×400.)

Peritoneal function and UF volume were partially preserved in the ENA group. TGFβ_1 was undetectable in the dialysate of the control and ENA groups, while a very high level of TGFβ_1 (298 ± 96 pg/mL) was detected in the Dx group. In the ENA group, CA125 levels (0.94 ± 1.3 U/mL) were higher than in the control (0.35 ± 0.5 U/mL) and Dx groups (0.11 ± 0.2 U/mL) ($p < 0.05$). This indicates that there was less mesothelial remodeling in the hypertonic Dx group than in the other two groups.

In the histological examination, while the peritoneal membrane in the control group appeared as a single layer of MCs over the muscle tissue, the peritoneum was thicker and edematous in the hypertonic Dx group (4.7 ± 0.4 μ vs 77.8 ± 7.2 μ, $p < 0.001$). Semiquantitative histopathological findings are summarized in Table 2. Mononuclear cell infiltration, fibroblastic activity, neovascularization, and interstitial edema were more prominent in the Dx group than in the ENA group (Table 3). Compared to the control group, peritoneal thickness was significantly increased in the ENA group (4.7 ± 0.4 μ vs 38 ± 5.04 μ, $p < 0.001$) but not as much as in the hypertonic Dx group (77.8 ± 7.2 μ (Figure 1).

DISCUSSION

Pharmacological maneuver of peritoneal solute transport is currently used to evaluate the functional properties and prevent peritoneal fibrosis, augmenting the viability of the peritoneal membrane.

Our study shows that hypertonic PD solutions play a major role in inducing peritoneal fibrosis and UFF. The rats exposed to hypertonic PD solutions showed structural deterioration in the peritoneal membrane, including increased thickness, neovascularization, inflammatory cell infiltration, and fibrosis. In addition, marked alterations in peritoneal function, such as reduced UF volume and increased solute transport and protein loss, were present, resembling type I UFF. In that situation, the increased solute permeability made fluid removal during dialysis difficult.

The functional alterations induced by hypertonic PD solutions were partially prevented by enalapril treatment. Indeed, this drug reduced the damage to the peritoneal structure. Although vascularity, fibrosis, and inflammatory cell infiltration were less in the ENA group than in the Dx group, peritoneal thickness was more prominent in the ENA group than that in the control group.

The mechanisms by which hypertonic PD solutions induce these structural and functional alterations in the peritoneum are not completely understood. In the present study, we showed that high dextrose concentration did increase TGFβ_1 expression. This

Downloaded from http://www.pdiconnect.com/ by guest on February 23, 2013

confirms earlier reports (18,19) in which it was proposed that $TGF\beta_1$ plays a central role in mediating the biological effect of high glucose dialysate on peritoneal MCs leading to peritoneal fibrosis (19).

Given the role of ANG II in promoting $TGF\beta_1$ expression, and the fibrogenic potential of this cytokine, interference with ANG II formation or its ANG I-receptor binding by existing pharmacological strategies (*i.e.*, ACE-I or AT1Ra) needs to be addressed.

Fibrotic diseases are characterized by the accumulation of extracellular matrix. $TGF\beta_1$ overexpression is shown to be responsible for this accumulation through its actions in inducing production of extracellular matrix, inhibiting its degradation, and increasing integrin expression, resulting in matrix deposition (8). To our knowledge, this is the first study showing that enalapril prevents $TGF\beta_1$ overexpression and ameliorates peritoneal morphology and function. We noticed that peritoneal thickness could not be completely prevented, although $TGF\beta_1$ overexpression was completely inhibited. We hypothesize that when production of $TGF\beta_1$ is interfered with, synthesis of extracellular matrix components caused by other cytokines, such as IL-1 or tumor necrosis factor-α, may become evident (8,20).

Several important properties of MCs enable them to maintain physiological homeostasis of the peritoneum through several actions, including (1) regulation of solute and water transport; (2) modulation of peritoneal microcirculation by secretion of vasodilators, such as PGE2 and nitric oxide, or vasoconstrictors, such as endothelin; (3) regulation of peritoneal fibrinolysis; (4) production and remodeling of extracellular matrix; and (5) a local antibacterial defense mechanism (21).

These cells express CA125; its concentration in peritoneal effluent represents MC mass or MC turnover in stable PD patients (3). A gradual loss of MC mass induced by chronic exposure to high dextrose concentration occurs in most patients during PD (22).

It has been suggested that high glucose-induced suppression of MC growth and regeneration is mediated by $TGF\beta_1$ overexpression (19). In the present study, CA125 concentration in peritoneal effluent was lower in the hypertonic Dx group, but higher in the ENA and control groups. This indicates that mesothelial remodeling was less in the hypertonic Dx group than in the other two groups. In contrast to a previous study (23), our results indicate that daily infusion of 10 mL physiological saline into the peritoneal cavity for 4 weeks is not harmful to the peritoneum. This can be explained by the low volume used. This finding shows that enalapril can preserve mesothelial mass and can increase MC regeneration by inhibiting $TGF\beta_1$ overexpression.

In conclusion, our results clearly show that commercially available hypertonic solutions can cause morphological and functional alterations in rat peritoneum under the experimental circumstances that we used, and can increase the production of $TGF\beta_1$. Peritoneal function can be partially preserved by inhibiting $TGF\beta_1$ overexpression with enalapril. Mesothelial cell regeneration and remodeling can also be maintained. Although the production of $TGF\beta_1$ was significantly inhibited, peritoneal thickness could not be prevented completely. This finding shows that several mechanisms other than $TGF\beta_1$ expression may be responsible for this feature.

ACKNOWLEDGMENT

This study was presented as an oral communication at the Fourth European Peritoneal Dialysis Meeting.

REFERENCES

1. Selgas R, Fernandez-Reyes M-J, Bosque E, Bajo M-A, Borrego F, Jimenez C, *et al.* Functional longevity of the human peritoneum: how long is continuous peritoneal dialysis possible? Results of a prospective median long-term study. *Am J Kidney Dis* 1994; 23:64–73.
2. Davies SJ, Bryan J, Philips L, Russel GI. Longitudinal changes in peritoneal kinetics: the effect of peritoneal dialysis and peritonitis. *Nephrol Dial Trans-*

Downloaded from http://www.pdiconnect.com/ by guest on February 23, 2013

Peritoneal Dialysis International

plant 1996; 11:448–56.
3. Heimbürger O, Waniewski J, Werynski A, Tranæus A, Lindholm B. Peritoneal transport in CAPD patients with permanent loss of ultrafiltration capacity. *Kidney Int* 1990; 38:495–506.
4. Honda K, Nitta K, Horita S, Yumura W, Nihei H. Morphological changes in the peritoneal vasculature of patients on CAPD with ultrafiltration failure. *Nephron* 1996; 72:171–6.
5. Rubin J, Herrera GA, Collins D. An autopsy study of the peritoneal cavity from patients on continuous ambulatory peritoneal dialysis. *Am J Kidney Dis* 1991; 18:97–102.
6. Fracasso A, Baggio B, Ossi E, Del Prete D, Bonfante L, Bazzato G, *et al*. Glycosaminoglycans prevent the functional and morphological peritoneal derangement in an experimental model of peritoneal fibrosis. *Am J Kidney Dis* 1999; 33:105–10.
7. Honda K, Nitta K, Horita S, Yumura W, Nihei H, Nagai R, *et al*. Accumulation of advanced glycation end products in the peritoneal vasculature of continuous ambulatory peritoneal dialysis patients with low ultra-filtration. *Nephrol Dial Transplant* 1999; 14:1541–9.
8. Chaimovitz C. Peritoneal dialysis. *Kidney Int* 1994; 45:1226–40.
9. Dobbie JW. Pathogenesis of peritoneal fibrosing syndromes (sclerosing peritonitis) in peritoneal dialysis. *Perit Dial Int* 1992; 12:14–27.
10. Border WA, Noble NA. TGFβ in kidney fibrosis: a target for gene therapy. *Kidney Int* 1997; 51:1388–96.
11. Peters H, Border WA, Noble NA. Targeting TGFβ overexpression in renal disease: maximizing the antifibrotic action of angiotensin II blockade. *Kidney Int* 1998; 54:1570–80.
12. Sun Y, Ratajska A, Zhou G, Weber KT. Angiotensin converting enzyme and myocardial fibrosis in the rat receiving angiotensin II or aldosterone. *J Lab Clin Med* 1993; 122:395–403.
13. Ratajska A, Campbell SE, Cleutjens JPM, Weber KT. Angiotensin II and structural remodeling of coronary vessels in rats. *J Lab Clin Med* 1994; 124:408–15.
14. Thomas DW, Hoffman MD. Identification of macrophage receptor for angiotensin: a potential role in antigen uptake for T lymphocyte responses? *J Immunol* 1984; 132:2807–12.
15. Juncos LI, Carrosco Deunas S, Cornejo SJ, Broglia CA, Cejas H. Long term enalapril and hydrochlorothiazide in radiation nephritis. *Nephron* 1993; 64:249–55.
16. Burdmann EA, Andoh TF, Nast CC, Evan A, Connors BA, Coffman TM, *et al*. Prevention of experimental cyclosporin-induced interstitial fibrosis by losartan and enalapril. *Am J Physiol* 1995; 269:F491–9.
17. Gronbaek H, Vogel I, Osterby R, Lancranjan I, Flyvbjerg A, Orskov H. Effect of octreotide, captopril or insulin on renal changes and UAE in long-term experimental diabetes. *Kidney Int* 1998; 53:173–80.
18. Kang DH, Hong YS, Lim HJ, Choi JH, Han DS, Yoon K. High glucose solution and spent dialysate stimulate the synthesis of transforming growth factor-β_1 of human peritoneal mesothelial cells: effect of cytokine costimulation. *Perit Dial Int* 1999; 19:221–30.
19. Kumano K, Schiller-Moran B, Hjelle JT, Moran JE. Effect of high glucose levels on cell proliferation and TGF-β expression in cultured rat mesothelial cell (Abstract). *J Am Soc Nephrol* 1993; 4:410.
20. Yang WS, Kim BS, Lee SK, Park JS, Kim SB. Interleukin-1β stimulates the production of extracellular matrix in cultured human peritoneal mesothelial cells. *Perit Dial Int* 1999; 19:211–20.
21. Nagy JA. Peritoneal membrane morphology and function. *Kidney Int Suppl* 1996; 50:S2–11.
22. Pannekeet MMH, Hiralali JK, Struijk DG, Krediet RT. Longitudinal follow-up CA125 in peritoneal effluent. *Kidney Int* 1997; 51:888–93.
23. Wang T, Heimbürger O, Qureshi AR, Waniewski J, Bergström J, Lindholm B. Physiological saline is not a biocompatible peritoneal dialysis solution. *Int J Artif Organs* 1999; 22:88–93.

Peritoneal Dialysis International Peritoneal Dialysis International Peritoneal Dialysis International

Downloaded from http://www.pdiconnect.com/ by guest on February 23, 2013