Effects of Mycophenolate Mofetil and Rapamycin on Peritoneal Fibrosis in an Experimental Model of Peritoneal Dialysis

Deneysel Periton Diyaliz Modelinde Mikofenolat Mofetil ve Rapamisinin Periton Fibrozisine Etkileri

ABSTRACT

OBJECTIVE: This study aimed to investigate the effect of mycophenolate mofetil and rapamycin on peritoneal function and morphology in a rat model of experimental peritoneal dialysis.

MATERIAL and METHODS: A total of 60 male Albino rats (Wistar strain) were equally divided into four groups as follows: Mycophenolate mofetil group (MMFG), rapamycin group (RAPG) and no-treatment group (NTG) receiving 15 mL 3.86% glucose solution, and control group (CG) receiving daily intraperitoneal injection of 15 mL normal saline. Mycophenolate mofetil and rapamycin were administered orally via daily gastric gavage in MMFG and RAPG. We conducted one-hour peritoneal equilibration tests at 8 weeks, measuring ultrafiltration, serum and dialysate growth factors and cytokines. Peritoneal membrane morphology was examined under the light microscope.

RESULTS: Compared to controls, levels of serum and dialysate vascular endothelial growth factor were significantly higher in NTG ($p<0.05$), but they did not differ significantly in MMFG and RAPG ($p>0.05$). Net ultrafiltration was significantly higher in MMFG than in NTG ($p<0.05$). A significant decline was detected in the number of mesothelial, inflammatory and fibroblast cells and peritoneal membrane thickness in MMFG, as compared with NTG.

CONCLUSION: We conclude that peritoneal functions such as ultrafiltration capacity can be protected by treatment with MMF and RAP, as they produce a membrane-protective effect in peritoneal dialysis.

KEY WORDS: Experimental peritoneal dialysis, Mycophenolate mofetil, Peritoneal fibrosis, Rapamycin, Rat

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ÖZ

AMAÇ: Çalışmanın amacı deneysel bir sıçan periton diyaliz modelinde mikofenolat mofetil ve rapamisinin periton fonksiyonu ve morfolojisi üzerine etkisini araştırmaktır.

GEREÇ ve YÖNTEMLER: Wistar-Albino 60 sıçan 4 eşit gruba ayrıldı: Mikofenolat mofetil grubu (MMFG), rapamisin grubu (RAPG) ve tedavi almayan grubu (NTG) %3,86 glikozlu solüsyonundan 15 mL ve kontrol grubu (CG) normal izotonik sıvıdan 15 mL günde tek doz intraperitoneal enjeksiyonla aldı. MMFG ve RAPG günlük gastrik gavaja sırasıyla mikofenolat mofetil ve rapamisin aldılar. Sekiz hafta sonunda 1 saatlik bir periton eşitleme testi yapıldı. Ultrafiltrasyon, serum ve diyalizatta büyüme faktörleri ve sitokinler ölçüldü. Periton membran morfolojisi ışık mikroskopisi ile değerlendirildi.

BULGULAR: Serum ve diyalizat vasküler endotelyal büyüme faktör düzeyleri CG ile karşılaştırıldığında NTG da anlamlı oranda daha yüksek saptandı ($p<0,05$), fakat MMFG ve RAPG gruplarında anlamlı fark saptanmadı ($p>0,05$). Net ultrafiltrasyon NTG'la karşılaştırıldığında MMFG'da anlamlı oranda daha yüksekti ($p<0,05$). NTG ile karşılaştırıldığında mezotelyal, inflamatuvar ve fibroblast hücre sayısı ile periton membran kalınlığı MMFG'da anlamlı oranda daha düşük saptandı.

SONUÇ: Ultrafiltrasyon gibi periton fonksiyonlarının MMF ve RAP ile korunduğu ve bu ilaçların periton diyalizinde periton membranını koruyucu etkiye sahip oldukları sonucuna vardık.

ANAHTAR SÖZCÜKLER: Deneysel periton diyalizi, Mikofenolat mofetil, Peritoneal fibrozis, Rapamisin, Sıçan

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INTRODUCTION

It has been argued that peritoneal dialysis (PD), if administered on a long-term basis with dialysis solutions containing glucose, may result in morphological and functional changes in the peritoneum (1, 2). Associated with high technique failure rates in long-term PD, peritoneal fibrosis (PF) is a serious issue leading to large number of drop outs from PD. A wide range of factors such as various injuries, exposure to bio-incompatible PD solutions, uremic syndrome, peritonitis, and chronic inflammation have been argued to trigger peritoneal fibrosis. When peritoneal membrane is constantly exposed to high-glucose solutions, it suffers several impairments that include development of interstitial fibrosis, reduplicated mesothelial basement membrane, thickening of blood vessels, neoangiogenesis, and hyalinized blood vessel media (3, 4). On the other hand, findings of the most recent research imply that new fibroblasts might stem from mesothelial cells (MC) that are locally converted by epithelial-mesenchymal transition (EMT) during inflammatory/repair responses triggered by peritoneal dialysis, thus blaming mesothelial cells for such injury to the peritoneal membrane. Enlarged vascular surface area might lead to significant increase in small solute transport, which results in the loss of peritoneal ultrafiltration (UF) capacity (1, 2).

While the mechanism causing these changes is yet to be well established, previous research suggested that PF might be associated with endothelial growth factors and cytokines released by mesothelial cells and peritoneal macrophages. Furthermore, MCs might be causing PF by secreting extracellular matrix macromolecules containing collagen, fibronectin, laminin and proteoglycans. Vascular endothelial growth factor (VEGF), transforming growth factor-beta 1 (TGF-β1), basic fibroblast growth factor (bFGF) and nitric oxide (NO) are those prominent mediator cytokines and endothelial growth factors that are involved in the development peritoneal fibrosis (5-7).

VEGF plays a critical regulatory role in angiogenesis and vascular permeability. Expression studies on this growth factor have demonstrated that long-term exposure to glucose-based PD solution induces a clear upregulation of VEGF expression (8, 9, 10). Such studies have also detected abundant amounts of VEGF in dialysate, which is directly associated with small solute permeability and impaired UF capacity (11). Therefore, with reference to other angiogenic conditions, upregulation of VEGF expression is argued to have a potential to induce vascular proliferation in the peritoneal membrane in patients receiving long-term PD treatment (12).

Multiple lines of evidence show that TGF-β1 is the key cytokine in the development of fibrosis. TGF-β1 enhances the synthesis of extracellular matrix proteins, such as collagen types I, III, and IV, laminin and fibronectin (13, 14). Mesothelial cells produce the fibrogenic cytokine TGF-β1 after stimulation by high-concentration glucose solutions (15, 16), that suggests a relationship between high-concentration glucose solutions

and induction of PF through TGF-β1. Evidence from clinical studies indicates TGF-β1 presence in the peritoneum, which is up-regulated during episodes of peritonitis. Elevated levels of TGF-β1 in dialysate concentrations have been shown to be associated with poorer outcomes in PD patients (8, 9, 17).

Like TGF-β and platelet-derived growth factor, bFGF (basic fibroblast growth factor) is also a member of large classical cytokine family that was shown to play an important part in the manifestation of most fibrotic diseases (18), with mitogenic properties effective in several cell types such as fibroblasts, endothelial and smooth muscle cells. In addition, bFGF appears an effective agent that induces formation of mesenchymal cells and new blood vessels (19). While certain studies detected bFGF in dialysis effluent collected from PD patients, the true impact of this growth factor on patients treated with PD is yet to be determined (20-22).

Nitric oxide is known to play an exceptional part in structural and functional alterations of the peritoneal membrane. Some researchers have assumed that NO might be directly influencing collagen formation, while inversely regulating TGF-β (23). Still, the role of this free radical in the development of fibrosis is more complicated than it appears. It has also been argued that nitric oxide promotes a protective mechanism against fibrosis during repair process in wound healing, whereas constant inhibition of nitric oxide synthase (NOS) activity eventually results in exacerbation of experimental fibrosis in various animal models (24).

A new immunosuppressive drug, mycophenolate mofetil (MMF) is a potent and specific inhibitor of inosine monophosphate dehydroxygenase (IMPDH). This novel drug induces a marked decline in lymphocyte proliferation, causing inhibition of collagen deposition and fibroblast proliferation, while interfering mesangial and vascular smooth muscle cells. MMF also causes progressive reduction in cytokines and growth factors derived from lymphocytes and macrophages (25). Shihab et al. suggested that MMF treatment caused considerable improvement in afferent arteriolopathy, while decreasing TGF-β1 expression (26). A promising therapeutic agent, rapamycin has shown very potent immunosuppressive and antifibrotic properties in experimental renal ischemia-reperfusion (27). Rapamycin has also been found to inhibit cell multiplication stimulated by growth factors, which includes proliferation of fibroblasts, endothelial and smooth muscle cells, effectively suppressing intimal hyperplasia in experimental vascular injury models (28). These actions may be mediated by restriction of epithelial-mesenchymal transition for tubular and tumor cells.

Rapamycin and MMF therapies have been reported to decrease proliferation and production of extracellular matrix through proximal tubular epithelial cells and myofibroblasts, which further corroborates the antifibrotic efficacy of such immunosuppressive drugs (29-31). Our hypothesis was that MMF and rapamycin might modulate the EMT of mesothelial cells,

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thus reducing its deleterious effects on peritoneal membrane. Therefore, such activities of MMF and rapamycin may translate into a major beneficial effect in preventing peritoneal fibrosis. In the current study, we performed an experimental PD model to investigate the effect of MMF and rapamycin on peritoneal function and PF induced by hypertonic PD solutions in rats.

MATERIALS and METHODS

Our study included a sample of sixty (60) non-uremic, male Albino rats (Wistar strain), whose weights ranged from 210 to 260g. For housing of the rats, polycarbonate cages were used, where they were given a standard laboratory animal diet as well as free access to water. The rats were kept under light-dark cycles of 12 hours, with temperature maintained at 24°C. Prior to any research protocols, a written approval regarding the study design was obtained from the animal ethics committee of the Cukurova University Medical School. The rats were equally divided into four groups and dialyzed with the following peritoneal dialysis regimens: MMFG (n=15) received 15 mL 3.86% glucose PD solution IP plus MMF; RAPG (n=15) received 15 mL 3.86% glucose PD solution IP plus RAP; NTG (n=15) received only 15 mL 3.86% glucose PD solution IP; and the CG (n=15) received only 15 mL isotonic saline IP.

The dosages of MMF (Hoffman-La Roche, Basel, Switzerland) and RAP (Wyeth-Pharmaceuticals, UK) were adjusted consistent with the previous experimental studies (32-35). MMF and RAP were suspended in sterile water to a final concentration of 10 mg/mL and 0.1 mg/mL, and then administered by gastric gavage at a dose of 10 mg/kg/d and 0.15 mg/kg/d respectively. Since these drugs are not fully water soluble, the resultant mixture was agitated vigorously to obtain a uniform suspension. This suspension was immediately administered by gastric gavage. We injected daily PD solutions into the lower left abdominal cavities for 8 weeks. 4 rats in MMFG, 5 rats in RAPG, 6 rats in NTG, and 4 rats in CG were excluded from the study because of gastroenteritis or a subcutaneous abscess due to leakage of inoculums into the subcutaneous tissue.

At 8 weeks, one-hour peritoneal equilibration tests (PET) were carried out, where we slowly injected 20 mL 2.27% dextrose PD solution into peritoneal cavity via a 22G catheter. Following the injections, the animals were allowed normal ambulation, with unlimited water access. After one hour, the rats were anesthetized with an intravenous dose of ketamine at 60 mg per kg of body weight. A midline incision was created for PD catheter insertion into the peritoneal cavity, preventing possible dialysate leaks from peritoneal cavity, and subsequently we collected samples of dialysate. At the end of the experiment, terminal blood collection through direct cardiac puncture was performed under anesthesia, and then the research animals were humanely euthanized through decapitation. Blood and dialysate samples were centrifuged immediately (+4 °C, 5000 rpm for 10 minutes), with removal of undesirable supernatant; and then the samples were stored at -80°C until the analysis time.

The peritoneal cavity was opened to check the residual fluid, and to obtain samples of visceral and parietal peritoneum from locations far from the injection site (right site of lower anterior abdominal wall).

Net UF volume was measured as the difference between the instilled and drained dialysate. Blood and dialysate urea nitrogen, total protein and glucose were measured using standard methods. Peritoneal transport characteristics were determined using dialysate-to-plasma (D/P) concentration ratios of urea nitrogen and total protein and D_1/D_0 glucose. D_1/D_0 glucose was determined by the ratio of glucose concentration in the drained dialysate to the unused 2.27% PD solution. Blood and dialysate VEGF, TGF-β1 and bFGF levels were measured using a commercially available enzyme-linked immunosorbent assay kit (Immunoassay kit, R&D Systems, Abingdon, OX, UK), and total NO level was measured using a commercially available enzyme-linked immunosorbent assay kit (Total NO assay kit, R&D Systems, Abingdon, OX, UK).

The specimens of the peritoneal membrane sections were fixed in 10% formaldehyde solution in phosphate-buffered saline (PBS) at room temperature and processed routinely for light microscopy. 5 μM thick paraffin-embedded tissue sections were obtained for histological examination and stained with hematoxylin-eosin and Mason trichome dyes. Selected sections were also stained by immunoperoxidase methods for collagen III and collagen IV (Dako; Glostrup, Denmark). The sections were examined under the light microscope (Olympus, BX50, Japan) by two pathologists without any indication of the group they belonged to. The fibrotic scores for the histological sections were determined as the sum of the following seven parameters: (a) number and reactivity of mesothelial cells; (b) change in basal lamina; (c) presence of inflammation, (d) submesothelial edema, (e) fibroblastic activity and fibrosis, (f) vascularization, (g) peritoneal thickness; and they were semi-quantitatively scored.

Mesothelial cells were categorized as normal cells (flat) and reactive cells (cubic transformation). Thickness of basal laminae was classified as either normal or thick. Fibrosis status was assessed as follows: no fibrosis, early (edema and lacy collagen pattern) fibrosis, medium (lacy and mature collagen), or late (mature collagen fibrils) fibrosis. Mesothelial cells were counted in 5 different areas, with mean values calculated (cells/high power filled at x 400 magnification) and classified as normal, decreased and increased. Semi-quantitative scoring for inflammation, fibroblastic activity, and neovascularization was conducted through counting of the mononuclear cells, fibroblasts, and capillaries per high power field (at x400 magnification). Peritoneal interstitial thickness was measured from the inner surface of the muscle to the mesothelium by an ocular micrometer. The values were calculated as the mean of 5 different areas measured by the same researcher as previously described (36).

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Statistical analysis: All research data were presented as mean ± SEM. The statistical analyses included ANOVA, unpaired t-test, and Mann-Whitney U tests. For all test results, a p value less than 0.05 (p<0.05) was considered statistically significant.

RESULTS

Levels of serum and dialysate VEGF, TGF-β1, bFGF and NO were shown in Table I. Serum VEGF levels were significantly higher in NTG than in CG (p<0.05), with no significant difference in MMFG and RAPG as compared with CG (p>0.05). Dialysate VEGF levels were significantly higher in NTG than those of CG (p<0.05), but they were not significantly different in MMFG and RAPG if compared with CG (p>0.05). Serum and dialysate TGF-β1, bFGF and NO levels showed no significant difference in all groups (p>0.05).

Functional findings of the study were summarized in Table II. Net UF was significantly lower in the rats in NTG and RAPG as compared to controls (p<0.05), while the mean UF in MMFG was not significantly different from that of CG (p>0.05). In addition, the mean UF was significantly higher in MMFG as compared with NTG (p<0.05). Mean D/P_{BUN} did not differ significantly in all four groups. However, the mean D/P protein level was significantly higher in NTG and RAPG than in CG (p<0.05), with no difference between MMFG and CG (p>0.05). In MMFG, the mean D/P protein levels were significantly lower than those of NTG (p<0.05). A significantly sharper decline in D_1/D_0 glucose ratio was observed in NTG, as compared with those of CG and MMFG (p<0.05), but it did not differ significantly from RAPG (p>0.05).

Table I: Levels of serum and dialysate VEGF, TGF-β1, bFGF and total NO.

Parameters	Groups			
	MMFG (n=11) Mean ± SD	**RAPG (n=10)** Mean ± SD	**NTG (n=9)** Mean ± SD	**CG(n=11)** Mean ± SD
Serum VEGF (pg/ml)	45.34 ± 6.86	44.35 ± 8.46	54.36 ± 25.25	43.26 ± 5.99
Serum TGF-β1 (pg/ml)	69.73 ± 25.40	79.39 ± 18.17	79.20 ± 10.52	71.48 ± 11.87
Serum bFGF (pg/ml)	8.31 ± 1.24	9.84 ± 2.95	9.62 ± 2.86	8.65 ± 1.32
Serum total NO (µMol/L)	140.75 ± 61.37	144.84 ± 114.98	168.88 ± 123.73	162.78 ± 144.22
Dialysate VEGF[b] (pg/ml)	40.05 ± 20.60	38.00 ± 10.19	47.73 ± 30.63	31.99 ± 3.60
Dialysate TGF-β1 (pg/ml)	46.38 ± 2.34	48.00 ± 3.07	53.60 ± 6.59	49.05 ± 5.66
Dialysate bFGF (pg/ml)	7.97 ± 2.01	7.65 ± 1.25	9.00 ± 1.57	8.27 ± 0.67
Dialysate total NO (µMol/L)	17.25 ± 6.34	17.55 ± 4.23	17.39 ± 3.36	17.20 ± 6.07

[a]p<0.05 in NTG vs. CG, [b]p<0.05 in NTG vs. CG, and RAPG vs. NTG. p>0.05 between other groups.
MMFG: Mycophenolate mofetil group, **RAPG:** Rapamycin group, **NTG:** No treatment group, **CG:** Control group

Table II: Functional findings of study (results of PET)

Parameters	Groups			
	MMFG (n=11) Mean ± SD	**RAPG (n=10)** Mean ± SD	**NTG (n=9)** Mean ± SD	**CG (n=11)** Mean ± SD
Ultrafiltration (mL)[a]	12.36 ± 2.42	6.00 ± 1.33	5.02 ± 1.60	11.64 ± 1.31
D/P_{BUN}	0.70 ± 0.37	0.78 ± 0.23	0.79 ± 0.17	0.83 ± 0.13
D/P protein[b]	0.08 ± 0.06	0.17 ± 0.11	0.25 ± 0.13	0.06 ± 0.04
D_1/D_0 glucose[c]	0.37 ± 0.14	0.27 ± 0.17	0.21 ± 0.08	0.36 ± 0.06

[a]p<0.05 in NTG vs. CG and in RAPG vs. CG and in MMFG vs. NTG. [b]p<0.05 in NTG vs. CG and in RAPG vs. CG and in MMFG vs. NTG. [c]p<0.05 in NTG vs. CG and in MMFG vs. NTG.
D/P: Dialysate to plasma ratio, D_1/D_0: Glucose concentration in the drained dialysate to the unused fluid, **MMFG:** Mycophenolate mofetil group, **RAPG:** Rapamycin group, **NTG:** No treatment group, **CG:** Control group

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Semi-quantitative histopathological findings were summarized in Table III. While mesothelial cell morphology was normal in 36% (4/11) of MMFG and in 91% (10/11) of CG, it was reactive in 89% (8/9) of NTG group, and in 100% (10/10) of RAPG. Basal lamina was normal in MMFG (91% was normal, 9% was thick), RAPG (80% was normal, 20% was thick) and CG (100% was normal) but thicker in NTG (11% was normal, 89% was thick). Submesothelial edema was more prominently increased in RAPG and NTG, although sustained no increase in MMFG and CG. No interstitial fibrosis was detected in controls, but late fibrosis was seen only in NTG rats. In other groups, interstitial fibrosis was only observed as early fibrosis, with no late fibrosis.

Morphological results for peritoneum in rats under different treatments and controls were shown in Table IV and Figure 1A-D. The number of mesothelial cells was significantly higher in RAPG and NTG when compared with CG (p<0.05). While there is no difference between the MMFG and controls (p>0.05), it was found that number of mesothelial cells in MMFG were significantly lower than NTG (p<0.05). Inflammation cells (especially lymphocytes) in MMFG and RAPG showed no significant difference from NTG (p>0.05). Fibroblast cells were significantly lower in MMFG when compared with NTG (p<0.05). As compared with controls, peritoneal membrane thickness was not significantly different in MMFG (p>0.05), but it was increased in RAPG and NTG (p<0.05).

Table III: Semiquantitative data for mesothelial cells and submesothelial area

Peritoneal membrane	MMFG Cells (%)	RAPG Cells (%)	NTG Cells (%)	CG Cells (%)
MC morphology				
Normal	4 (36)	0 (0)	1 (11)	10 (91)
Reactive	7 (64)	10 (100)	8 (89)	1 (9)
Basal lamina				
Nomal	10 (91)	8 (80)	1 (11)	11 (100)
Thick	1 (9)	2 (20)	8 (89)	0 (0)
Submesothelial edema				
Absent	4 (36)	0 (0)	1 (11)	11 (100)
Present	7 (64)	10 (100)	8 (89)	0 (0)
Interstitial fibrosis				
Absent	4 (36)	2 (20)	1 (11)	11 (100)
Early	6 (55)	6 (60)	1 (11)	0 (0)
Middle	1 (9)	2 (20)	3 (33)	0 (0)
Late	0 (0)	0 (0)	4 (45)	0 (0)

MC: Mesothelial cell. **MMFG:** Mycophenolate mofetil group, **RAPG:** Rapamycin group, **NTG:** No treatment group, **CG:** Control group

Table IV: Morphological results for peritoneum in rats under different treatments

Parameters	Groups			
	MMFG (n=11) Mean ± SD	RAPG (n=10) Mean ± SD	NTG (n=9) Mean ± SD	CG (n=11) Mean ± SD
[a]Count of mesothelial cells	13.35 ± 5.31	21.32 ± 6.90	20.91 ± 5.05	9.36 ± 3.25
Count of inflammation cells[a]	7.22 ± 6.89	11.52 ± 8.95	16.09 ± 11.59	0.13 ± 0.40
Neoangiogenesis[c]	5.16 ± 3.15	6.90 ± 3.90	9.51 ± 6.93	0.50 ± 1.06
[b]Count of fibroblast cells	6.73 ± 3.16	10.96 ± 5.70	12.96 ± 5.86	4.70 ± 2.20
[c]PM thickness (μm)	97.19 ± 84.95	250.96 ± 56.02	232.29 ± 110.12	39.92 ± 18.47

[a]p<0.05 in RAPG vs. CG, in NTG vs. CG and in MMFG vs. NTG; [b]p<0.05 in MMFG vs. NTG; [c]p<0.05 in RAPG vs. CG and in NTG vs. CG; p>0.05 between other groups.
MMFG: Mycophenolate mofetil group, **RAPG:** Rapamycin group, **NTG:** No treatment group, **CG:** Control group.

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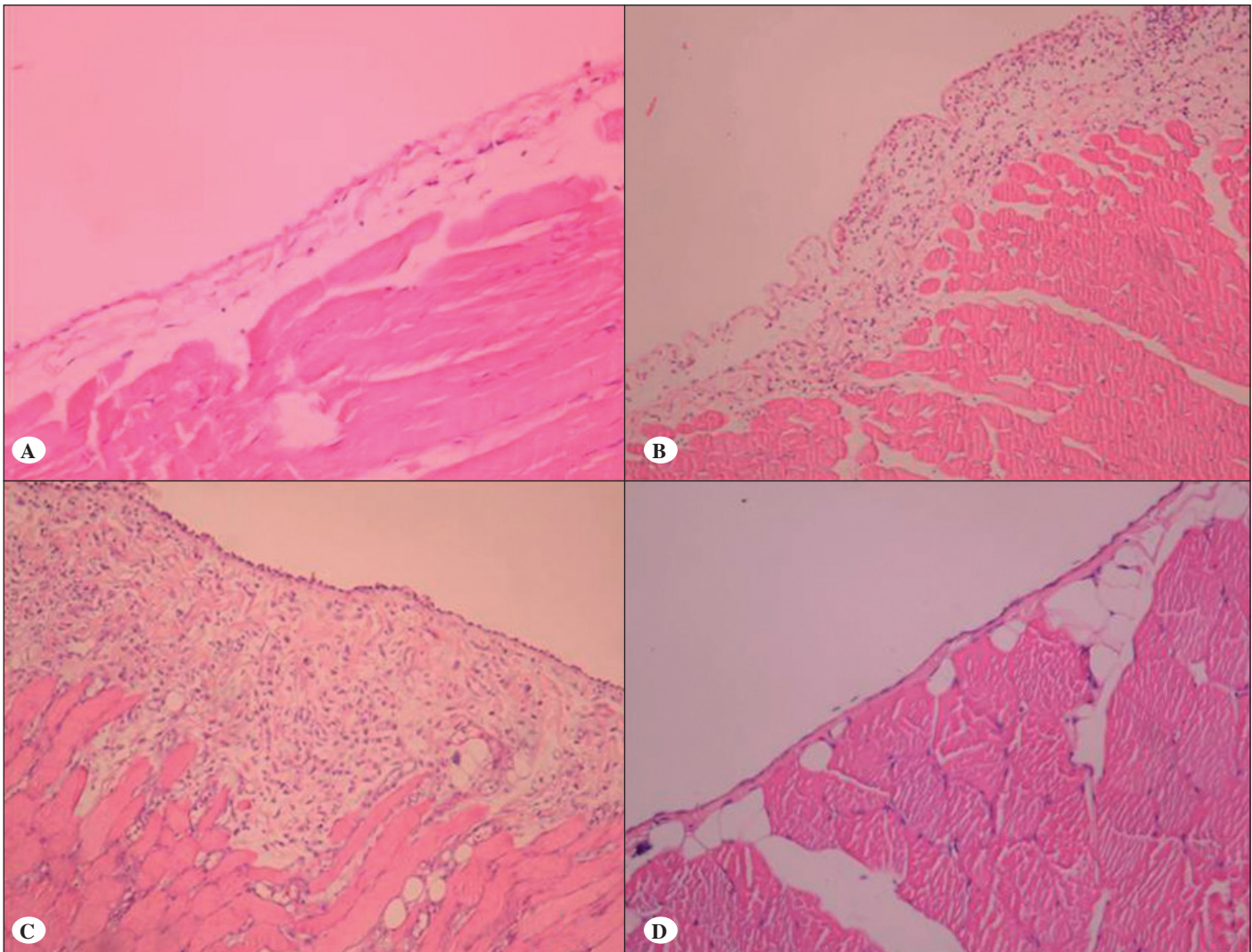Fibrosis in an Experimental Model of Peritoneal Dialysis

Figure 1: Morphology of the peritoneum in MMF (A), RAP (B), NTG (C) and control groups (D) (Hematoxylin-eosin, x 400).

DISCUSSION

The bioincompatibility of dialysis solutions has been suggested as one of the reasons for the changes in the structure and function of the peritoneum during long-term PD (1, 2). Previous research in this field, using animal models, has examined the efficacy of treatment with ACE (angiotensin-converting enzyme) inhibitors, angiotensin receptor blockers (ARBs), octreotide and corticosteroids on peritoneal alterations that are induced by hypertonic PD solutions (36-38). There are very limited studies, no matter experimental or clinical, that attempt to investigate and report the effects of MMF and RAP in fibrotic diseases (25, 26, 30, 39, 40). Besides, previous work in this field has so managed to produce little direct evidence that would suggest additional biocompatible dialysis solutions can preserve peritoneal function.

It has been well established that vascular endothelial growth factor plays a vital part in both fibrosis and neoangiogenesis, the two basic mechanisms in the pathogenesis of PF. An endothelial cell mitogen and potent angiogenic factor, VEGF is produced in the peritoneum during PD treatment with glucose-based PD solutions, and then it undergoes endothelial upregulation along the peritoneal blood vessels in long-term PD (8, 10, 11). This implies that VEGF activation may play a role during the progression of PF and that inhibition of VEGF may reduce fibrosis as well as angiogenesis. If we assume that MMF and RAP have potential to inhibit serum and dialysate VEGF activity, we can speculate that MMF and RAP therapies may prevent PF progression via inhibition of EMT. In our study, serum VEGF levels of both MMF and RAP groups were similar to those of controls, whereas in NTG group this level was mildly increased, though significantly higher than controls. Dialysate VEGF levels in NTG were higher when compared with controls, while RAPG had significantly lower levels than no-treatment group. In addition, dialysate VEGF levels in MMFG were not different

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from those of controls. Our findings suggest that secretion of VEGF may be inhibited by MMF and RAP in peritoneum. If we assume that RAP and MMF are be capable of inhibiting serum and dialysate VEGF activity, we can speculate that RAP and MMF may inhibit PF in patients receiving PD treatment.

Limited evidence from clinical studies indicates the presence of TGF-β1 in the peritoneum, which is upregulated during episodes of peritonitis. The human studies in this field have provided sufficient evidence that TGF-β1 is a major player in peritoneal fibrosis. Margetts et al. reported that active TGF-β1 induced a prolonged submesothelial fibrosis that might be in connection with elevated expression of α-smooth muscle actin-positive myofibroblasts in the interstitium (41, 42). Increased dialysate concentrations of TGF-β1 were reported to correlate with poorer outcomes in PD patients, while they were thought to stimulate myofibroblastic response in mesothelial cells, which triggers peritoneal fibrosis (17, 43). VEGF is also closely related to TGF-β1 activity during PD. Another study employing TGF-β1 gene transfer reported that peritoneal overexpression of TGF-β1 escalated VEGF production, thus leading to hyper-vascularity (41, 42). Therefore, through direct and indirect mechanism via VEGF, TGF-β1 can be argued to exacerbate peritoneal fibrosis and angiogenesis. Since activated fibroblasts produce TGF-β1 and VEGF, they seem to be involved in neo-angiogenesis in a PD model. In our study, serum TGF-β1 levels were mildly decreased in MMFG, but there was no significant intergroup difference. In addition, dialysate TGF-β1 levels did not differ significantly in all groups. These findings suggest that MMF and RAP may not inhibit the secretion of serum and dialysate TGF-β1.

Basic fibroblast growth factor is known to stimulate mitogenicity of several cell types such as fibroblasts, endothelial and smooth muscle cells, while promoting mesenchymal and vascular formation (12). In addition, bFGF has been shown to intensify multiplication of human peritoneal fibroblasts, with a much stronger impact than TGF-β1. In their studies, Fukasawa et al. and Beavis et al. stated that recombinant human FGF-basic led to a considerable increase in the reproduction of peritoneal fibroblasts (44, 45). In our study, we found no intergroup differences in terms of serum and dialysate bFGF levels, nor did we detect any effect of MMF and RAP on bFGF secretion.

Among its diverse functions, nitric oxide also plays a vital part in the regulation of vascular tone and reactivity, interacting with other growth factors to regulate tumor angiogenesis. Recent research conducted in peritoneum from humans and rats indicated that endothelial NOS underwent a considerable upregulation in cases of peritoneal inflammation or acute peritonitis (7, 46). Another study argued that nitric oxide might be protective of fibrosis, while constant inhibition of NOS exacerbated fibrosis in rats (24). In our study, total NO levels in serum and dialysate did not differ significantly between all rat groups. We supposed that MMF and RAP did not have an effect on the secretion of

NO in serum and dialysate. These results suggest that NO levels do not have any impact on peritoneal fibrosis in long-term PD patients.

This has been the first study to investigate the impact of both MMF and RAP on peritoneal function in an experimental PD model. MMF led to total renewal of all functional parameters of the peritoneum, including UF capacity, D1/D0 glucose and D/P protein (Table II). The results of this research showed that exposure to hypertonic PD solutions have major impacts on peritoneal function and fibrosis. The rats treated with rapamycin (RAPG), as well as those in the NTG, had significant changes in peritoneal function, including reduction in UF volume, higher ratio of dairy protein or protein loss, together with lower rates of D_1/D_0 glucose. While MMF and control groups were partly protected from such changes, the RAP and NT groups were affected.

In conclusion, our research findings have demonstrated that exposure to hypertonic PD solutions is a lead factor which triggers peritoneal membrane failure and peritoneal fibrosis. In this study, we have determined that MMF and RAP may have the potential to partially inhibit the secretion of VEGF in serum and dialysate, although ineffective in the prevention of TGF-β1, NO and bFGF secretion. We have concluded that peritoneal functions such as ultrafiltration capacity can be protected by treatment with MMF and RAP, as they produce a membrane-protective effect in PD patients. Such effects might partly be associated with inhibition of VEGF, as well as another pathway such as MMF-induced antiproliferative effects. Further studies with larger sample sizes and longer treatment periods are therefore needed to confirm the efficiency of MMF and RAP in the prevention of peritoneal fibrosis.

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