

EDITORIAL

THE RENIN–ANGIOTENSIN SYSTEM AND PERITONEAL DIALYSIS

During continuous ambulatory peritoneal dialysis (CAPD), various morphological changes take place in the peritoneum, including mesothelial denudation, interstitial fibrosis, neovascularization, and such vascular alterations as the replication of basement membrane and fibrosis and hyalinization of the vascular wall (1–8). Among the suggested causes of these histological and functional alterations are recurrent peritonitis, the influence of plasticizers and advanced glycosylation end-products, and response to the nonphysiological nature of peritoneal dialysis (PD) solutions, in particular its high glucose content, hypertonicity, lactate, and low pH (8–10). Although the mechanisms underlying these alterations are not completely understood, it has been shown that growth factors and cytokines secreted by mesothelial cells (MC) and macrophages are associated with peritoneal fibrosis (11). Dobbie suggested that the initiating events that lead to peritoneal fibrosis and injury to MC are chronic irritation by dialysis solutions and severe or prolonged peritonitis (12). Damaged MC 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.

A key mediator of ECM accumulation in fibrotic diseases, $TGF\beta_1$ 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 matrix metalloproteinase (MMP) expression and increased production of protease inhibitors, such as tissue inhibitor of matrix metalloproteinase (TIMP) and plasminogen activator inhibitor type 1; and (3) increased synthesis of integrins (13). *In vitro*, $TGF\beta_1$ orchestrates sequential cellular events that include loss of epithelial adhesion, F-actin reorganization, disruption of basement membrane integrity, and enhanced cell migration and

invasion (14). Reduction of $TGF\beta_1$ overexpression decreases pathological matrix accumulation.

The $TGF\beta$ superfamily regulates cell proliferation, differentiation, adhesion, and apoptosis, and thus controls embryonic development, tissue recycling, and wound repair (15). $TGF\beta$ binds directly to the $TGF\beta$ receptors II (a constitutively active transmembrane serine/threonine kinase that recruits $TGF\beta$ receptors) and I, and phosphorylates one or more substrates to initiate a signal cascade such as that of Smad proteins and integrin-linked kinase (14).

Angiotensin II (ANG II), the main peptide of the renin–angiotensin system (RAS), is an oligopeptide of eight amino acids, formed from its precursor, angiotensinogen, by a series of two enzymatic cleavages. The effects of ANG II are mediated by binding to specific ANG II receptors: AT1 and AT2. Vascular and renal tubular actions are mediated primarily by AT1 receptors. The effects of AT2 receptors are less well understood; they may play a role of regulation of cell proliferation in the arterial wall. It is now clear, however, that there are extra-renal RAS and that ANG II can be synthesized at a variety of sites, including the kidney, vascular endothelium, adrenal gland, and brain. It is presumed that local ANG II production is important for the regulation of local processes that are activated by local factors such as prostaglandin, nitric oxide, and endothelin. Angiotensin II is considered a growth factor that regulates cell proliferation, apoptosis, and fibrosis. In models of renal injury, blockage of ANG II action via angiotensin-converting-enzyme inhibitors (ACE-I) or angiotensin-receptor blockers (ARBs) decreases proteinuria, inflammatory-cell infiltration, fibrosis, and gene expression of matrix proteins and growth factors (13,16,17). RAS blockers are commonly used in the treatment of hypertension and proteinuria in humans (18).

Angiotensin II stimulates macrophages and fibroblast-like cells to secrete $TG\beta_1$. A perivascular/

interstitial fibrosis, for instance, accompanies chronic elevation of either circulating ANG II or aldosterone (19) and, in the case of ANG II, occurs in response to abnormal vascular permeability and escape of macromolecules (20). ANG II also regulates the synthesis of proinflammatory cytokines (tumor necrosis factor α , interleukin-6) and chemokines (monocyte chemoattractant protein-1) in the kidney (21). Importantly, ANG II may have a direct effect on downstream modulators of collagen metabolism, that is, MMPs, TIMP (22), and collagen tissue growth factor (23). The MMPs seem to play an increasingly prominent role as inflammatory mediators in the regulation of ECM proteins and cell proliferation via the TGF β_1 pathway (20). There is also some evidence of TGF β_1 -independent pathways of ANG II-induced fibrosis (24).

It has been shown that activated macrophages isolated from peritoneal exudates (formed after instillation of mineral oil into the peritoneal space) express ANG II receptors (25).

Peritoneal matrix accumulation is characteristic of peritoneal fibrosis. Continuous ambulatory PD patients who had persistent TGF β_1 in their effluent had an increased risk of peritoneal fibrosis. We know that TGF β_1 regulates cell proliferation, differentiation, adhesion, and apoptosis, and thus controls tissue recycling and wound repair. TGF β_1 binds directly to TGF β receptors and phosphorylates one or more substrates to initiate a signal cascade, such as that of Smad proteins. Seven human Smad proteins have been identified. Smad1 is thought to be a mediator of bone morphogenic protein signaling, whereas Smad2 and Smad3 are responsible for TGF β and activin signaling (26).

Recent studies have demonstrated increased evidence of ANG II involvement in the regulation of angiogenic cytokines, such as vascular endothelial growth factor (VEGF) and angiopoietin, and their receptors (27). ANG II plays a role in promoting angiogenesis. Rizkalla *et al.* clearly demonstrated that ANG II is a major stimulus of angiogenic cytokines and their receptors. Specifically, both AT1- and AT2-receptor-blockades antagonize the actions of ANG II on the expression of VEGF and angiopoietin (28).

Some *in vitro* studies have investigated the effects of RAS on peritoneum. Yao *et al.*, who investigated the role of the TGF β_1 -Smad pathway in preventing peritoneal fibrosis in human peritoneal mesothelial cells (HPMC), showed that dialysis with high glucose upregulated the expression of Smad2 and Smad4, whereas conditions of high osmolarity (as with mannitol) did not affect the TGF β_1 -Smad signaling pathway. They also showed that ARB (losartan) inhibited the concentration of TGF β_1 and the expression of Smad2. They concluded that ARBs might represent a

possible way to prevent and treat fibrosis of the peritoneum in long-term PD patients (29).

Noh *et al.* investigated the possibility that an independent RAS was operating in HPMC and examined the effects of glucose on it. They demonstrated that HPMC have an independent RAS and that high glucose concentrations upregulate it. ANG II mediates high-glucose-induced fibronectin secretion by HPMC, and blockade of RAS by the ACE-I imidapril may have a therapeutic benefit in long-term PD (30).

Using an encapsulating peritoneal sclerosis model in mice, Sawada *et al.* also showed that the ACE-I quinapril ameliorates peritoneal fibrosis (31).

Abe *et al.* investigated the role of bone-marrow-derived cells in the formation of peritoneal fibrotic tissue and the importance of ANG II type 1 receptor in bone-marrow-derived cells in the progression of peritoneal fibrosis in a chlorhexidine-induced fibrosis model in mice. They showed that ANG II acting via AT1 receptor in bone-marrow-derived cells might play a pivotal role in the progression of peritoneal fibrosis by elevating the level of growth factors and chemokines (32).

Imai *et al.* investigated changes in the expression of aquaporins (AQPs) in the peritoneum in rats and examined the effects of the RAS on the expression of AQPs with and without an ACE-I (benazepril) or an ARB (valsartan). Treatment with benazepril or valsartan significantly suppressed the expression of AQP-1 and AQP-4. Those authors suggest that the RAS plays an important role in the regulation of water transport in the peritoneum (33).

Using animal models, we investigated the effect of ACE-I or ARBs, or a combination of both, on peritoneal alterations induced by hypertonic PD solutions. Thus, in a rat model of once-daily injection of 3.86% glucose PD fluid for 4 weeks, we found that (1) oral administration of enalapril ameliorates changes in peritoneal function and morphology (34), (2) oral administration of lisinopril (an ACE-I) and valsartan (an ARB) have similar beneficial effects on peritoneal function and morphology (35), and (3) oral administration of quinapril plus valsartan has no synergistic effects on peritoneal alterations induced by 3.86% glucose PD solutions (36). On histological examination, we found less cell infiltration and less vascularization in all treated groups. According to results of Rizkalla *et al.* (28), inhibition of angiogenesis by ANG II blockage might partially explain the functional benefits observed in our experiments (34). Based on these findings, we concluded that RAS blockade ameliorates hypertonic PD solution-induced peritoneal injury by inhibiting the overexpression of cytokines (*i.e.*, TGF β_1 and VEGF), and that ACE-I and/or ARBs might preserve the viability of peritoneum in CAPD patients over the long term.

In the clinical setting, in a pilot study, Agraharkar *et al.* investigated the effects of RAS blockage on peritoneal protein loss in PD patients. They concluded that RAS blockage did not reduce protein loss into the peritoneal fluid during dialysis (37).

ACE-I and ARBs may have an important if indirect role in PD by affecting the rate of decline of residual renal function in PD patients, especially in those with diabetic nephropathy (38,39) and chronic proteinuric nephropathy (40,41). In a randomized study of 60 patients on PD, Li *et al.* investigated the effect of ACE-I on residual renal function. Over a 12-month period, the average residual glomerular filtration rate (GFR) declined by 2.07 mL/minute/1.73 m² in the ramipril (5 mg/day) group versus 3.00 mL/minute/1.73 m² in the control group ($p = 0.03$). At 12 months, 14 patients in the ramipril group ($n = 30$) and 22 in the control group ($n = 30$) became anuric. Those authors concluded that the ACE-I ramipril might reduce the rate of decline of residual renal function in PD patients (42). Similar studies in patients with diabetic nephropathy and other chronic proteinuric nephropathies found that the benefits of ramipril seem to be independent of the level of systemic blood pressure.

In another study, Kikuta *et al.* examined the effects of valsartan on residual renal function in CAPD patients for 3 months to 2 years after the start of dialysis. Valsartan significantly retarded the progressive decline in residual renal function (residual GFR 4.1 to 3.2 vs 4.1 to 2.2 mL/min/1.73 m²) (43).

Ample evidence from *in vitro* experiments shows that the beneficial effect of ACE-I therapy is related to the paracrine effect of ANG II (accumulation of extracellular matrix) rather than its hemodynamic effect (44).

The plasma levels of ACE are modulated by ACE gene polymorphism. The polymorphism was classified into three genotypes: II, ID, and DD. An association between progressive renal disease and the DD genotype was found in a prospective follow-up study of 56 patients with type II diabetes (45) and in patients with type I diabetes (46). There are a few studies in the literature that clarify the role of genes related to ACE in PD patients. In one of them, Nishina, from Japan, investigated polymorphism of the ACE gene in 60 PD patients and 50 patients undergoing hemodialysis (47). The author found a significant difference in allele frequency between normal subjects ($n = 100$) (I = 0.63, D = 0.37) and dialysis patients ($n = 110$) (I = 0.46, D = 0.54, $p < 0.001$). The mean plasma ACE activity and the mean rate of decrease in residual urinary volume were higher with the DD genotype ($p < 0.05$). The mean rate of decrease in residual urinary volume was positively correlated with plasma ACE activity ($r =$

0.133 89, $p < 0.02$). The mean cardiothoracic ratio was higher in PD patients with the DD genotype. The author concluded that PD patients with the DD genotype lost residual renal function more rapidly and had a larger heart than patients with the other genotypes (47).

In another study, Wang *et al.* investigated cardiac hypertrophy and remodeling in relation to ACE and angiotensinogen genotypes in Chinese PD patients. They found that polymorphism of the angiotensinogen M235T gene, but not the ACE I/D gene, is associated with greater left ventricular mass and relative wall thickness, indicating more concentric left ventricular hypertrophy, in Chinese PD patients (48).

Finally, Varagunam *et al.* found a significant difference in erythropoietin requirement in the II/ID group compared to the DD group. They concluded that ACE genotype has predictive value when determining erythropoietin dosage, as the II/ID genotype may be associated with a suboptimal response (49).

What we learned from these three studies is that gene polymorphism is also important for evaluating PD patients, and we need to clarify the relation between gene polymorphism and transport status and developing peritoneal fibrosis.

CONCLUSION

The evidence to date suggests that the renin-angiotensin system plays a role in high-glucose-induced peritoneal injury by regulating proinflammatory cytokines, increasing aquaporin expression, and increasing progression of fibrosis. Evidence is emerging that ACE-I and ARBs may have a beneficial effect in preventing the long-term alterations of peritoneal membrane induced by high glucose solutions, and possibly decreasing the rate of decline of residual renal function in these patients. Well-designed, randomized controlled trials are required to determine whether the renin-angiotensin system and its blockade will play an important role in the long-term outcome of PD patients.

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