Atrial natriuretic peptide ameliorates peritoneal fibrosis in rat peritonitis model

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Abstract

Background. Atrial natriuretic peptide (ANP) was recently reported to ameliorate fibrosis in the heart and experimental renal diseases and vascular thickening after balloon injury. Peritoneal fibrosis is an important complication of long-term peritoneal dialysis, and peritonitis is a factor in its onset. In the present study, we investigated the effects of ANP in a rat peritonitis-induced peritoneal fibrosis model.

Methods. As pretreatment, an osmotic pump containing vehicle (saline) or ANP (0.15 or 0.3 μg/min) was inserted through the carotid vein in male Sprague–Dawley rats. ANP or saline was continuously infused using the osmotic pump. Three days after administration of ANP or saline, rats underwent peritoneal scraping in a blind manner and were sacrificed on Day 14. The effects of ANP were evaluated based on peritoneal thickness, immunohistochemistry and real-time polymerase chain reaction. In each experiment, we evaluated messenger RNA (mRNA) expression of the ANP receptor natriuretic peptide receptor A (NPR-A) in the peritoneum after scraping. The effects of ANP were also studied in cultured peritoneal fibroblasts and mesothelial cells.

Results. We observed a significant increase in NPR-A mRNA in the peritoneum. Peritoneal thickness increased with time and peaked on Day 14, but ANP significantly reduced peritoneal thickness. Parameters such as number of macrophages and CD-31-positive vessels and expression of type III collagen/transforming growth factor-β/plasminogen activator inhibitor-1 (PAI-1)/connective tissue growth factor (CTGF) were significantly suppressed by ANP. In cultured peritoneal fibroblasts and mesothelial cells, ANP suppressed angiotensin II-induced upregulation of CTGF and PAI-1.

Conclusions. Our results suggest that ANP is useful in preventing inflammation-induced peritoneal fibrosis.

Keywords: ANP; CTGF; peritoneal fibrosis; renin-angiotensin system; TGF-β

Introduction

The characteristic feature of chronic peritoneal damage in peritoneal dialysis (PD) treatment is decreased ultrafiltration capacity associated with submesothelial fibrosis,
ANP ameliorates peritoneal fibrosis

The decrease in ultrafiltration capacity seen after prolonged PD often results in its discontinuation [2, 3]. Both retrospective and prospective studies have found that peritonitis impairs the ultrafiltration capacity of the peritoneal membrane [4], and is an important risk factor for ultrafiltration failure [4, 5]. The degree of associated peritonitis inflammation determines changes in peritoneal function [5, 6]. Histologically, acute peritoneal inflammation can cause morphological damage to the peritoneum [7–9]. These reports indicate that peritonitis and inflammation can lead to structural changes and to dysfunction of the peritoneal membrane. Therefore, we hypothesized that anti-fibrotic agents, together with antibiotics, may be useful in PD patients with peritonitis to prevent deterioration of peritoneal membrane function.

Recent reports have shown that the renin-angiotensin-aldosterone system (RAAS) is involved in the peritoneal fibrosis in PD patients and in animal models [10–12]. Angiotensin II concentration in PD effluent is significantly higher at the onset of infectious peritonitis than in normal PD patients [13]. Angiotensin II receptor blockade (ARB) and angiotensin- converting enzyme inhibitor (ACE) reportedly reduced the thickness of the submesothelial compact zone and the number of CD-31-positive vessels in a chlorhexidine gluconate-induced peritoneal fibrosis model [14, 15]. In human studies, ARB, valsartan, not only preserved residual renal function but also increased peritoneal creatinine clearance [16]. In addition to systemic RAAS, peritoneal mesothelial cells have been shown to constitutively express angiotensin-converting enzyme (ACE), the angiotensin II type 1 receptor (AT1-R) and angiotensin II type 2 receptor (AT2-R) and mineralocorticoid receptor, which indicates that local RAAS is present in the peritoneum [11, 12]. These findings indicate that RAAS plays a role in the progression of peritoneal fibrosis and deterioration of ultrafiltration function in PD patients.

Natriuretic peptides (NPs) consist of atrial NP (ANP), brain NP (BNP) and C-type NP (CNP). ANP and BNP are selective ligands for natriuretic peptide receptor A (NPR-A), and CNP is a specific ligand for natriuretic peptide receptor B (NPR-B) [17]. Evidence suggesting that ANP/BNP prevents cardiac hypertrophy and fibrosis independent from blood pressure is now accumulating. Both cardiac myocytes and cardiac fibroblasts have been found to express NPR-A and NPR-B [17–19]. In studies using cultured neonatal myocytes and fibroblasts, exogenous administration of both ANP and BNP confirmed that they have antihypertrophic and anti-fibrotic functions [18, 20]. In addition, experiments in ANP- or BNP-deficient mice and NPR-A KO mice have demonstrated that inhibition of the ANP/BNP/NPR-A signaling pathway induces cardiac hypertrophy and/or fibrotic changes [21–26], while exogenous ANP inhibits collagen synthesis and DNA synthesis in cardiac fibroblasts [18, 27, 28] and ANP increases levels of cGMP and inhibits RAAS and prepro-endothelin-1 expression induced by angiotensin II in cardiac fibroblasts [18, 29, 30].

In this study, although the etiology differs from that of bacterial peritonitis, we explored the therapeutic potential of human ANP (h-ANP) in a rat model of peritoneal fibrosis induced by mechanical scraping, an acute experimental nonbacterial form of peritonitis, which we recently reported [12].

Materials and methods

Agents

Angiotensin II was purchased from the Peptide Institute (Osaka, Japan). Carperitide, recombinant h-ANP, was provided by Daiichi Asubio Pharma Co., Ltd. (Osaka, Japan).

Animals and experimental design

All animal studies were carried out in accordance with the Animal Experimentation Guidelines of Nagoya University Graduate School of Medicine (Nagoya, Japan). Seven-week-old male Sprague–Dawley (SD) rats (Japan SLC, Hamamatsu, Japan) initially weighing 210–230 g were used throughout the study. The animals were maintained under conventional laboratory conditions and were given free access to food and water. At the beginning of the experiments, rats were incised at the abdominal midline under anesthesia with diethyl ether, and the right parietal peritoneum were mechanically scraped for 60 s with the tops of 15–ml centrifuge tubes, as described previously [12]. After scraping, abdominal incisions were sutured, and after surgery, rats had free access to 1% NaCl in tap water.

In order to determine the expression of AT1-R, NPR-A, ACE and connective tissue growth factor (CTGF) messenger RNA (mRNA) in this model, rats were sacrificed on Days 0 (control, before scraping), 3, 7 and 14 (n = 4 at each time point). Prior to sacrifice, animals were anesthetized with diethyl ether and parietal peritoneal samples were obtained for light microscopy and immunohistochemistry.

In blocking studies, five groups were used. Scraped rats were randomly assigned to three groups: vehicle (Group I, n = 11), h-ANP (0.15 μg/min) (Group II, n = 7) or h-ANP (0.30 μg/min) (Group III, n = 7). Sham rats treated with h-ANP (0.15 μg/min) (Group IV, n = 6) and sham rats without treatment (Group V, n = 6) were used as controls. As pretreatment, an osmotic pump (Alzet model 2KL4; ALZET, Cupertino, CA) containing h-ANP or saline was inserted through the carotid vein, and the pump body was placed under the back skin in the male SD rats. h-ANP and saline were continuously infused with the osmotic pump. Three days after administration of h-ANP or saline, the peritonea of rats were scraped in a blind manner, and rats were sacrificed at Day 14. Blood pressure was measured by tail-cuff plethysmograph (BP-98A; Softron, Tokyo, Japan) once per week. Prior to sacrifice, animals were anesthetized with diethyl ether and blood samples were obtained. Subsequently, parietal peritoneal samples were procured. Peritoneal thickness, immunohistochemistry for cytokeratin, α-smooth muscle actin (α-SMA), transforming growth factor-β (TGF-β), plasminogen activator inhibitor-1 (PAI-1), CTGF, type III collagen, ED-1 and CD-31 mRNA expression were examined in harvested samples.

Measurement of plasma h-ANP concentration

At sacrifice, blood samples were rapidly taken from all rats and immediately centrifuged to isolate plasma in order to measure the concentration of h-ANP. h-ANP concentrations were measured using an immunoradiometric assay kit (hANP M02 kit; Shionogi, Osaka, Japan).

Histology and immunohistochemistry

Peritoneal tissue was processed for routine histology and immunohistology, as described previously [12, 31–33]. Part of the parietal peritoneal tissue was fixed in 10% buffered formalin and embedded in paraffin using conventional techniques. Sections (3 μm) were stained with hematoxylin and eosin. Another tissue sample was embedded in OCT compound (Sakura Finetechnical, Tokyo, Japan) and frozen in liquid nitrogen for immunostaining. Sections (4 μm) were cut with a cryostat, air-dried and fixed in acetone at room temperature for 10 min. Endogenous peroxidase activity was inhibited using 0.1% NaN3 and 0.3% hydrogen peroxide in PBS and non-specific protein-binding sites were blocked with 10% normal goat serum. Sections were then incubated
with rabbit anti-type III collagen antibody (Cosmo Bio, Tokyo, Japan), mouse anti-rat monocyte/macrophage antibody (ED-1; BMA Biomedicals AG, August, Switzerland), mouse anti-α-SMA antibody (1A4; Dako, Glostrup, Denmark), mouse anti-cytokeratin antibody (Dako), rabbit anti-TGF-β1, 2, 3 antibody (Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-PAI-1 antibody (Santa Cruz), rabbit anti-CTGF antibody (Torrey Pines Bioslabs, Houston, TX) or anti-rat CD-31 antibody (BD Bioscience, San Jose, CA), followed by a conjugate of polyclonal goat anti-rabbit IgG antibody or anti-mouse IgG antibody and horseradish peroxidase-labeled polymer (Histofine Simple Stain; Nichirei, Tokyo, Japan) as a secondary reagent. Enzyme activity was detected using a 3,3′-diaminobenzidine tetrahydrochloride liquid system or 3-amin-9-ethyl-carbazole (Dako) [12]. To detect cytoketatin, immunofluorescence studies were performed.

Morphological analysis
In order to assess the extent of peritoneal thickening, the submesothelial compact zone was identified as the membrane area extending from the surface mesothelium down to the upper limit of the muscular tissues. We measured peritoneal thickness at 12 random sites (6 sites in the central area and 6 sites in the lateral area) using a Zeiss Z1 microscope and Axiovision Windows software version 4.4 (Carl Zeiss, Oberkoehen, Germany), and mean thickness was calculated in the total, central and lateral sites, respectively [12]. The cytoketatin-positive mesothelial cell layer relative to total peritoneal surface length was calculated [12]. The numbers of ED-1-positive cells and CD-31-positive vessels were counted in 10 random 750 μm submesothelial areas. The α-SMA and type III collagen-positive area of the submesothelial compact zone was measured using MetaMorph 6.3 image analysis software (Universal Imaging Co., West Chester, PA). Peritoneal TGF-β1, PAI-1 and CTGF expression was analyzed and semiquantitatively classified into four groups: 0, no staining; 1, mild; 2, moderate and 3, pronounced staining. For each peritoneal tissue, ‘peritoneal expression score’ was assessed [12] and the mean of individual scores was calculated for the untreated h-ANP 0.15 μg/min and h-ANP 0.30 μg/min groups. Morphometric analyses were performed by two investigators blinded to the source of samples.

Cell culture study
Primary cultures of rat peritoneal fibroblasts were established from normal SD rat peritoneum as described previously [34]. Briefly, normal parietal peritoneal tissues were dissected from the surface of the peritonea and divided into 5-mm² pieces. These pieces were then transferred to tissue culture plates and were grown in complete medium containing Dulbecco’s Modified Eagle’s Medium (Sigma, Tokyo, Japan) supplemented with 5% fetal bovine serum (Sigma) in humidified air with 5% CO2 at 37°C. Modified Eagle Medium (Sigma, Tokyo, Japan) supplemented with 5% fetal bovine serum (Sigma) in humidified air with 5% CO2 at 37°C. Primary cultures of rat mesothelial cells were obtained from SD rat omentum, as described previously [35]. Fibroblasts or mesothelial cells from the second to fourth passages were used for experiments. Under subconfluent conditions, cells were washed twice with PBS, and culture medium was replaced with serum-free medium for 24 h in order to render cells quiescent. Subsequently, cultures were incubated with angiotensin II, in accordance with previous reports [36]. The concentration of angiotensin II selected for subsequent experiments was 10−8 mol/L. Cells were harvested at t = 0, 6, 12 and 24 h after incubation. Inhibition studies with h-ANP at 10−7 mol/L, which was based on previous reports [36, 37], were conducted on CTGF mRNA induction during incubation with 10−8 mol/L angiotensin II for 12 h.

RNA preparation from rat parietal peritoneum and rat fibroblasts
Rat parietal peritoneal tissues (30 mg) were immersed in RNAlater (Ambion, Austin, TX) for 1 day. The mixture was ground for 2 min with 5-mm tungsten carbide beads at a frequency of 27 Hz using a mixermill grinder according to the manufacturer’s instructions (Tissuelyser; Qiagen, Hilden, Germany). The ground solution was then centrifuged for 3 min at 10000 g in order to compact the debris and the supernatant was treated according to the manufacturer’s instructions. For rat tissues and fibroblasts, total RNA was extracted using the RNeasy Fibrous Tissue Mini Kit or RNeasy Mini Kit (Qiagen). RNA concentrations were estimated as described previously [12].

Polymerase chain reaction
We conducted polymerase chain reaction as described previously [12, 38]. A total of 1 μg of parietal peritoneal RNA or 2 μg of rat fibroblast RNA was then reverse transcribed. To validate gene expression changes, real-time polymerase chain reaction (RT-PCR) analysis was performed with an Applied Biosystems Prism 7500HT Sequence Detection System using TaqMan Gene Expression Assays according to the manufacturer’s specifications (Applied Biosystems Inc., Foster City, CA). TaqMan probes and primers for TGF-β1 (assay identification number Rn00572010_m1), Type III collagen (Rn01437683_m1), PAI-1 (Rn00561717_m1), NPR-A (Rn00561678_m1), AT1-R (Rn01435427_m1), ACE (Rn00561094_m1) and CTGF (Rn0073960_g1) and 18S ribosomal RNA (4326317E) were assay-on-demand gene expression products (Applied Biosystems). As an endogenous control, 18S ribosomal RNA (18S rRNA) was used. Thermal cycler conditions were as follows: 10 min at 95°C, followed by two-step PCR for 40 cycles of 95°C for 15 s and 60°C for 1 min. All reactions were performed in triplicate. Amplification data were analyzed using Applied Biosystems Sequence Detection Software version 1.3.1 (Applied Biosystems). To normalize the relative expression of the genes of interest against the 18S rRNA controls, standard curves were prepared for each gene and the 18S rRNA in each experiment [12].

Statistical analysis
Values are expressed as means ± SEs. Comparisons among groups of animals were performed by one-way analysis of variance, followed by Dunnett’s multiple comparison test. For statistical analysis of mRNA expression between h-ANP-treated and untreated groups, two-tailed Student’s t-test was performed. Comparisons of TGF-β1, CTGF and PAI-1 peritoneal expression between animals by immunohistochemistry were evaluated by Mann–Whitney test. Differences were considered to be statistically significant if P < 0.05. All analyses were performed using SPSS (Chicago, IL).

Results

Expression of AT1-R, NPR-A and CTGF expression in peritoneal fibrosis model induced by peritoneal scraping
The rat scraping model is characterized by acute-phase inflammation (on Day 3; Figure 1A, a and b) and late-phase peritoneal fibrosis (on Days 7–14; Figure 1A, c and d), as reported previously [12] (Figure 1). We examined mRNA expression in the peritoneum by real-time PCR in order to explore the possible involvement of RAS in this scraping model. AT1-R, ACE and NPR-A mRNA expression increased by 2.19-, 2.19- and 3.24-fold and peaked at Day 14, Day 7 and Day 7, respectively. In addition, CTGF mRNA peaked on Day 14 (Figure 1B). No PCR products were seen in the absence of complementary DNA from scraped peritonemum or in the absence of primers.

Effects of carperitide (h-ANP) on peritoneal fibrosis induced by scraping
We investigated the effects of h-ANP on the mechanical scraping-induced rat peritonitis model (Figure 3). Body weight was not significantly altered between the untreated and h-ANP-treated groups [before surgery: 292.1 ± 9.7 g (Group I) versus 287.9 ± 10.4 g (Groups II and III), 2 weeks after treatment: 349.2 ± 9.2 g versus 348.8 ± 9.7 g]. Systolic blood pressure was not significantly altered during experiments and was not affected by treatment (Table 1). Plasma h-ANP levels were significantly increased in the h-ANP administration group (Figure 2A). h-ANP administration resulted in a significant reduction
in peritoneal thickness [Group 1: 228.9 ± 18.3 μm; Group 2: 120.9 ± 22.6 μm (P < 0.01 versus Group 1); Group 3: 61.3 ± 6.0 μm (P < 0.001 versus Group 1)]. In the separate analysis of the central and lateral areas, significant reductions were seen in both the central (Figure 2C) and lateral areas (Figure 2D) when compared with the nontreatment group.

Semiquantitative and quantitative assessment of the effects of ANP

Up to 78% of the peritoneal surface was covered by cytokeratin-positive mesothelial cells in Groups I, II and III, and there were no differences between the three groups (Figure 4A). On semiquantitative assessment

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**Table 1.** Systolic blood pressure in untreated rats and in rats treated with ANP

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>0 Weeks, mean ± SE (mmHg)</th>
<th>1 Week, mean ± SE (mmHg)</th>
<th>2 Weeks, mean ± SE (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I scrape</td>
<td>Nontreatment</td>
<td>120.8 ± 6.8</td>
<td>119.3 ± 4.7</td>
<td>118.0 ± 5.9</td>
</tr>
<tr>
<td>Group II scrape</td>
<td>ANP (0.15 μg/min)</td>
<td>125.1 ± 3.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Group III scrape</td>
<td>ANP (0.3 μg/min)</td>
<td>117.8 ± 2.1</td>
<td>120.8 ± 3.4</td>
<td>118.0 ± 3.1</td>
</tr>
<tr>
<td>Group IV sham</td>
<td>ANP (0.15 μg/min)</td>
<td>113.5 ± 6.5</td>
<td>110.8 ± 2.9</td>
<td>120.2 ± 1.7</td>
</tr>
<tr>
<td>Group V sham</td>
<td>Nontreatment</td>
<td>118.5 ± 5.5</td>
<td>110.0 ± 2.9</td>
<td>120.2 ± 1.7</td>
</tr>
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*ND, not done.
by immunohistochemistry, α-SMA expression, Type III collagen deposition and CD-31-positive vessels were suppressed by administration of h-ANP at 0.15 and 0.30 µg/min (Figure 4A). The number of ED-1-positive macrophages was also significantly suppressed by 0.30 µg/min h-ANP (P < 0.05, Figure 4A). In addition, TGF-β (P < 0.005), PAI-1 (P < 0.05) and CTGF (P < 0.005) expression was significantly suppressed by 0.30 µg/min h-ANP (Figure 4A). To verify the results of immunohistochemical studies, quantitative analysis by real-time PCR for type III collagen, TGF-β, PAI-1 and CTGF mRNA in the peritoneal membrane was performed at Day 14. Upregulation of type III collagen (P < 0.05), TGF-β (P < 0.005), PAI-1 (P < 0.05) and CTGF (P < 0.05) mRNA was significantly inhibited by 0.30 µg/min h-ANP (Figure 4B). However, AT1-R, ACE and NPR-1 mRNA expression was not significantly different between the ANP treatment and non-treatment groups (data not shown) (Figures 3 and 4).
Effects of ANP on in vitro assay

The time course of CTGF and PAI-1 mRNA expression in response to angiotensin II treatment was studied in both fibroblasts and mesothelial cells (Figure 5). Samples were harvested after 0, 6, 12 and 24 h of exposure to angiotensin II. In peritoneal fibroblasts from normal peritonea, CTGF and PAI-1 mRNA expression was elevated and peaked at 24 h (Figure 5A and C). Angiotensin II-induced upregulation of CTGF and PAI-1 mRNA was significantly suppressed by $10^{-5}$ mol/L h-ANP (Figure 5B and D). In mesothelial cells, CTGF and PAI-1 mRNA were also elevated and peaked at 24 h after incubation of angiotensin II, which was significantly suppressed by h-ANP (Figure 5E–H).
Fig. 4. Continued.
ANP and BNP are circulating hormones of cardiac origin that bind to NPR-A, stimulating cGMP production and playing a key role in the regulation of intravascular blood volume and vascular tone [18]. Recent studies have suggested that the NP/cGMP system plays a counter-regulatory role against the rennin-angiotensin-aldosterone system, including ACE activity, rennin release, angiotensin II and aldosterone synthesis and TGF-β-mediated pathways [18, 30, 36, 39, 40]. In organs such as the kidney, the renoprotective effects of NPs have been reported in animal models of diabetic nephropathy, renal ablation and immune-mediated renal injury [41, 42].

We recently reported a new model of peritoneal fibrosis with acute inflammation induced by mechanical scraping [12]. The pathological stages of this model are (i) mesothelial exfoliation and exudation of fibrin on the surface of the membranes; (ii) leukocyte accumulation after initial influx of neutrophils and monocytes; (iii) resorption of fibrin and remesothelization; (iv) progressive fibrotic changes and increased vessel density associated with membrane dysfunction and (v) ~70% of the peritoneal surface area is covered with mesothelial cells at Day 14 [12]. This model resembles bacterial peritonitis in its leukocyte accumulation profile and progressive fibrotic changes with interaction between proinflammatory cytokines (monocyte chemoattractant protein-1) and pro-sclerotic growth factors (TGF-β and PAI-1) [12, 43–46]. Interestingly, we demonstrated the upregulation of NPR-A mRNA expression at Day 7 in the thickened peritoneum of this model, which raises the interesting possibility that NPs are able to modulate the structural remodeling of the peritoneum. We also observed the upregulation of ACE and

![Fig. 4. Analysis of immunohistochemical studies and real-time PCR in ANP-treated and untreated rats. (A) Ratio of cytokeratin-positive mesothelial cells on the peritoneal surface was not significantly different between h-ANP-treated (0.30 μg/min and 0.15 μg/min) and untreated rats. TGF-β, PAI-1 and CTGF expression was significantly suppressed by h-ANP (0.3 μg/min). Type III collagen deposition and α-SMA expression as analyzed by MetaMorph and number of ED-1-positive cells and CD-31-positive vessels were significantly reduced by treatment with h-ANP (0.3 μg/min). *P < 0.05, **P < 0.005, ###P < 0.001. All data were compared with untreated controls. (B) Quantitative analysis of type III collagen, TGF-β, PAI-1 and CTGF mRNA expression in peritoneum of ANP-treated and untreated rats. Increased expression of type III collagen, TGF-β, PAI-1 and CTGF mRNA was suppressed by h-ANP (0.3 μg/min). TGF-β, transforming growth factor-β; PAI-1, plasminogen activator inhibitor-1 CTGF; connective tissue growth factor. *P < 0.05, **P < 0.005, ###P < 0.001. All data were compared with untreated scraped rats.](image-url)
Fig. 5. Effects of h-ANP on angiotensin II-induced CTGF and PAI-1 mRNA expression in rat peritoneal fibroblasts and mesothelial cells; (A–D) Fibroblasts and (E–H) mesothelial cells. (A, B, E, F): CTGF mRNA expression, (C, D, G, H): PAI-1 mRNA expression, (A) Angiotensin II increased expression of CTGF mRNA by 2.2-fold at 24 h. (B) Angiotensin II-induced CTGF mRNA expression was suppressed by coincubation with h-ANP. (C) Angiotensin II increased expression of PAI-1 mRNA by 7.5-fold at 24 h. (D) Angiotensin II-induced PAI-1 mRNA expression was abolished by coincubation with h-ANP. (E) Angiotensin II increased expression of CTGF mRNA by 3.7-fold at 24 h. (F) Angiotensin II-induced CTGF mRNA expression was suppressed by coincubation with h-ANP. (G) Angiotensin II increased expression of PAI-1 mRNA by 6.1-fold at 24 h. (H) Angiotensin II-induced PAI-1 mRNA expression was inhibited by coincubation with h-ANP. All data are expressed as relative differences after normalization against 18S rRNA. Experiments were repeated at least three times. *P < 0.05, #P < 0.01 versus 0 h or control (n = 3 per group).
ANP ameliorates peritoneal fibrosis

Keiko Higashide, Ms Naoko Asano and Ms Yuriko Sawa (Department of Nephrology, Nagoya University, Nagoya) have been observed to express angiotensinogen, ACE, and mineralocorticoid receptor [11, 12, 47, 48]. On analysis of molecules that might be involved in peritoneal damage in this model, ANP was found to suppress inflammatory (macrophage infiltration) and fibrotic processes. In ANP-treated groups, expression of TGF-β, PAI-1 and CTGF, an important profibrotic growth factor that was recently reported to be elevated in PD patients with ultrafiltration failure [38], was significantly suppressed in association with type III collagen deposition. However, AT1-R, ACE and NPR-A mRNA expression did not significantly differ between the ANP treatment and non-treatment groups. In cultured rat peritoneal fibroblasts and mesothelial cells isolated from normal peritoneal tissues, angiotensin II increased CTGF and PAI-1 mRNA expression as in mesangial cells, tubular epithelial cells and vascular smooth muscle cells [49, 50]. Induction of CTGF and PAI-1 was effectively inhibited by ANP. These results support the notion that peritoneal fibroblasts and mesothelial cells are the target cells of ANP. Based on these findings, ANP may effectively suppress these cytokines and RAAS networks leading to prevention of peritoneal fibrosis in this model. ANP may also act on macrophages and inhibit the inflammatory responses, as reported previously [51], which may result in inhibition of fibrosis and angiogenesis.

Patients with acute myocardial infarction who were given ANP were recently reported to have lower infarct size, fewer reperfusion injuries and better outcomes than controls without prominent side effects [52, 29]. In the present study, we demonstrated that ANP acts on the damaged peritoneal tissues and is effective in preventing peritoneal membrane fibrosis in the rat peritonitis model, which indicates that ANP is promising for clinical use against peritoneal fibrosis in PD patients. Bacterial peritonitis remains an important factor in the induction of damage to the peritoneal membrane in PD patients. Clinical studies on NP together with antibiotics to prevent peritoneal fibrosis in PD patients with bacterial peritonitis are warranted in order to more definitively address the efficacy and safety of NP.

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Conflict of interest statement. None declared.

References


37. Lara-Castillo N, Zandi S, Nakao S et al. Atrial natriuretic peptide (CTGF/CCN2) is increased in peritoneal dialysis patients with high peritoneal solute transport rate. _Am J Physiol Renal Physiol_ 2010; 298: F721–F733

38. Mizzutani M, Ito Y, Mizuno M et al. Connective tissue growth factor (CTGF/CCN2) is increased in peritoneal dialysis patients with high peritoneal solute transport rate. _Am J Physiol Renal Physiol_ 2010; 298: F721–F733


42. Makino H, Mukoyama M, Mori K et al. Transgenic overexpression of brain natriuretic peptide prevents the progression of diabetic nephropathy in mice. _Diabetologia_ 2006; 49: 2514–2524


44. Hurst SM, Wilkinson TS, McLaughlin RM et al. IL-6 and its soluble receptor orchestrate a temporal switch in the pattern of leukocyte recruitment seen during acute inflammation. _Immunity_ 2001; 14: 705–714


48. Kiemer AK, Vollmar AM. The atrial natriuretic peptide regulates the in vivo fate of the inflammatory macrophage during the resolution of inflammation: inflammatory macrophages do not die locally, but emigrate to the draining lymph nodes. _J Immunol_ 1996; 157: 2577–2585


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56. Kiemer AK, Vollmar AM. The atrial natriuretic peptide regulates the in vivo fate of the inflammatory macrophage during the resolution of inflammation: inflammatory macrophages do not die locally, but emigrate to the draining lymph nodes. _J Immunol_ 1996; 157: 2577–2585