ROLE OF THE RENIN–ANGIOTENSIN SYSTEM IN THE PATHOGENESIS OF PERITONEAL FIBROSIS

Hidetomo Nakamoto,1 Hiroe Imai,2 Rie Fukushima,2 Yuji Ishida,2 Yasuhiro Yamanouchi,2 and Hiromichi Suzuki2

Department of General Internal Medicine1 and Department of Nephrology,2 Saitama Medical School, Saitama, Japan

Conclusions: Long-term intraperitoneal exposure to acidic dialysis solution produced features typical of EPS. Acidic dialysis solution induces activation of the peritoneal renin–angiotensin system and progression of peritoneal fibrosis. For the peritoneum undergoing peritoneal dialysis, ARB protects against progression of peritoneal fibrosis and peritoneal adhesions.

Encapsulating peritoneal sclerosis (EPS) is one of the most serious complications for the patient on peritoneal dialysis because it causes high morbidity and mortality from bowel obstruction (1–6). Several factors may induce this serious condition (peritonitis, acetate buffer, chlorhexidine, high-glucose, beta-blockers, plasticizers, and so on), although the mechanisms and pathogenesis remain unclear (3–5). Some investigators have reported that acidic dialysis solution may induce peritoneal fibrosis. To prevent development of EPS, it is important to reduce exposures to known risk factors.

Background: Although the effects of angiotensin type 1 receptor blocker (ARB) have been studied, little is known about ARBs in hypertensive patients undergoing dialysis. In the present study, we evaluated the effect of an ARB, olmesartan medoxomil (CS866), on the progression of peritoneal fibrosis in peritoneal dialysis by examining its effect in a model of peritoneal fibrosis in hypertensive rats.

Materials and Methods: We allocated 40 male Wistar rats with 2-kidney, 1-clip renovascular hypertension (2K1C-RVH) to 4 groups (each n = 10) that were dialyzed using various solutions for 42 days as follows:

- Group I—10 mL pH 3.5 dialysis solution containing 1.35% glucose
- Group II—10 mL pH 3.5 dialysis solution, plus oral administration of CS866 5 mg/kg daily
- Group III—10 mL pH 3.5 dialysis solution, plus oral administration of the calcium channel blocker (CCB) amlodipine 3 mg/kg daily
- Group IV—10 mL pH 7.0 dialysis solution

Dialysis solution was injected every day for 42 days.

Results: Treatment with CS866 and amlodipine induced a significant reduction of blood pressure in 2K1C-RVH rats. In rats treated with pH 3.5 dialysis solution, necropsy findings revealed features identical to those of encapsulating peritoneal sclerosis (EPS). The typical appearance was multiple surfaces covered with granulation tissue or fibrosic tissue or both. Multiple adhesions were present. Microscopic findings revealed that acidic dialysis solution induced peritoneal fibrosis and loss of mesothelium. Treatment with CS866 prevented the progression of peritoneal fibrosis and adhesions. However amlodipine did not improve the progression of peritoneal fibrosis and peritoneal adhesions. In CS866-treated rats, no signs of EPS were present.

Correspondence to: Hidetomo Nakamoto, Department of General Internal Medicine, Saitama Medical School, 38 Morohongo, Moroyama-machi, Iruma-gun, Saitama 350-0495 Japan.
nakamo_h@saitama-med.ac.jp

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The present study was designed to examine whether acidic dialysis solution induces peritoneal fibrosis. In addition, to investigate the role of the renin–angiotensin system in the progression of peritoneal fibrosis and the development of EPS, we examined the effect of an ARB in a model of EPS in hypertensive rats.

MATERIALS AND METHODS

The study was performed using 40 male Wistar rats (200 – 250 g) with 2-kidney, 1-clip renovascular hypertension (2K1C-RVH). All protocols were carried out in strict accordance with the animal care guidelines from the U.S. National Institutes of Health (12) and were approved by the Institutional Animal Care and Use Committee of Saitama Medical School.

Left renal artery banding and peritoneal catheter (PE50) insertion were carried out as previously described (13,14). In brief, a single dorsal skin incision exposed the left kidney. The artery of the rat was cleared as close as possible to the junction with the aorta, and a silver clip 0.25 mm in diameter was slipped around the artery. The end of the clip was pinched together to prevent it coming off. For the first 2 days after catheter insertion, the peritoneal cavity was rinsed with 10 mL saline. On the 3rd day, the 40 2K1C-RVH rats were divided into 4 groups (each \( n = 10 \)) and were infused with various solutions for 42 days:

- **Group I**—10 mL pH 3.5 dialysis solution containing 1.35% glucose (360 mOsm)
- **Group II**—10 mL pH 3.5 dialysis solution, plus daily oral administration of the ARB olmesartan medoxomil (CS866) 5 mg/kg
- **Group III**—10 mL pH 3.5 dialysis solution, plus daily oral administration of the calcium channel blocker amlodipine (AM) 3 mg/kg
- **Group IV**—10 mL pH 7.0 dialysis solution containing 1.35% glucose (360 mOsm).

Dialysis solution injection was performed every day for 42 days. To reduce pain, indomethacin 1 mg/kg was administered orally 1 hour before each intraperitoneal infusion of acidic and neutral solution.

During the experiment, the animals were weighed weekly. Systolic blood pressure and heart rate were taken weekly. Blood pressure was measured by the tail-cuff method previously described, and 5 consecutive measurements were made (15,16). On day 42, peritoneal samples were obtained, fixed using 10% formalin in phosphate-buffered saline, and embedded in paraffin. Sections were cut in a plane perpendicular to the mesothelial surface and stained with hematoxylin and eosin for examination by light microscopy.

STATISTICAL ANALYSIS

All results are expressed as mean ± standard error of the mean of the experiments. Statistical analysis was performed using StatView V. 5.0 on a Macintosh computer. Statistical analysis of blood pressure used two-way analysis of variance followed by the Scheffe \( F \)-test for paired data. Comparisons between groups were made using the Student \( t \)-test for unpaired data. Statistical significance was assumed at \( p \) value less than 0.05.

RESULTS

CHANGES IN SYSTOLIC BLOOD PRESSURE

Baseline blood pressure readings were approximately the same for the 4 groups. In rats treated with acidic dialysis solution, blood pressure gradually rose to 146 ± 6 mmHg at 42 days from 124 ± 6 mmHg at baseline. This gradual elevation in blood pressure was significantly reduced by the administration of AM (to 126 ± 6 mmHg, \( p < 0.05 \)) or CS866 (to 122 ± 5 mmHg, \( p < 0.05 \)). However, as compared with the blood pressure in rats treated with acidic solution, the blood pressure in rats treated with neutral solution showed no significant change.

NECROPSY FINDINGS IN PERITONEUM

In rats treated with pH 3.5 dialysis solution, necropsy findings showed evidence of EPS. The typical appearance was multiple surfaces covered with granulation tissue or fibrosic tissue or both. Peritoneal dullness or sclerosis was interpreted as evidence of gross thickening of peritoneum and was seen in all necropsies of rats injected with pH 3.5 dialysis solution. Adhesive peritonitis with multiple adhesions or complete encapsulation was seen in all necropsies of rats injected with pH 3.5 dialysis solution. Adhesive peritonitis with multiple adhesions or complete encapsulation was seen in all necropsies of rats injected with pH 3.5 dialysis solution. Partial adhesive peritonitis was seen in AM-treated rats, but treatment with CS866 completely prevented the progression of peritoneal fibrosis and adhesions in the peritoneum.

LIGHT MICROSCOPY FINDINGS IN PERITONEUM

After 42 days of intraperitoneal injections of dialysis solution, subserosal tissue thickening was observed to
became more significant as solution acidity increased (Figures 1 and 2). Among the solutions employed, pH 3.5 dialysis solution induced loss of the mesothelial layer, resulting in typical peritoneal fibrosis. This subserosal fibrous tissue contained spindle-shaped and rounded mononuclear cells (for example, fibroblasts and monocytes), and dense amorphous substances (for example, collagens and fibronectin). In addition, remarkable vascular sclerosis was observed in the subserosal tissue of the rats injected with pH 3.5 solution. By contrast, no subserosal tissue thickening was seen in rats injected with pH 7.0 dialysis solution.

The subserosal tissue thickening was significantly reduced in CS866-treated rats as compared with rats treated with AM. By contrast, in subserosal tissue thickening was slightly, but not significantly, reduced in rats treated with AM.

**DISCUSSION**

In the present study, long-term intraperitoneal administration of acidic dialysis solution induced severe peritoneal adhesions. The 2K1C-RVH rats treated with acidic dialysis solution showed a disease process and pathologic conditions similar to those of human EPS, including weight loss and peritoneal sclerosis and adhesions resulting in full-bowel EPS and cocoon formation. The typical appearance was multiple surfaces covered with granulation tissue or fibrosis tissue or both. A number of adhesions were present. Microscopic examination revealed peritoneal fibrosis and loss of mesothelium in the rats treated with acidic solution. In rats treated with neutral dialysis solution, no signs of EPS were present. These findings suggest that acidic dialysis solution induces the development of EPS, accompanied by activation of the renin–angiotensin system in the peritoneum. Neutral dialysis solution protected against the development of EPS.

The renin–angiotensin system is well known to be an important regulatory factor in natriuresis and diuresis in the kidney. Angiotensin II has several important endocrine, autocrine, and paracrine functions within the kidney. This peptide is integrally involved in autoregulation of the glomerular filtration rate in response to changes in renal perfusion pressure. Angiotensin II reduces urine volume and urinary excretion of sodium. Through its effects on renal hemodynamics and renal tubule epithelial function, it preserves extracellular fluid volume during states of acute hypovolemia and chronic salt depletion.

Angiotensin II is also well known to be an important regulatory factor in the kidney. Within the proximal tubule, it is produced in amounts 100 times those found in plasma (17). Angiotensin II within the proximal tubule is involved in Na⁺, water, and HCO₃⁻ reabsorption, and receptors for the peptide are present there. Binding studies have demonstrated that the proximal tubule predominantly contains AT1 receptors. Dietary salt restriction not only affects angiotensin II receptor regulation in angiotensin II target tissues, it also increases the density of angiotensin II receptors in the proximal tubule; increments in salt intake reduce the density of those receptors (17).
Previously, we demonstrated that the renin–angiotensin system plays a key role in the regulation of peritoneal function in rats on peritoneal dialysis (7). However, the long-term effects of the activation and inhibition of the renin–angiotensin system on peritoneal function and fibrosis remains unclear. We carried out the present study to investigate the role of the renin–angiotensin system in the progression of peritoneal fibrosis and adhesive peritonitis during CAPD.

In rats treated with neutral dialysis solution, we observed no signs of peritoneal fibrosis and EPS. Based on those data, we can speculate that the renin–angiotensin system in the peritoneum plays an important role in the progression of peritoneal fibrosis in rats treated with acidic solutions. Also in the present study, ARB treatment completely prevented the progression of peritoneal fibrosis and peritoneal adhesions in 2K1C-RVH rats treated with acidic dialysis solution. By contrast, a calcium channel blocker reduced blood pressure, but did not prevent the progression of peritoneal fibrosis. Based on those data, we conclude that the renin–angiotensin system plays an important role in the progression of peritoneal fibrosis in patients on peritoneal dialysis.

During CAPD, the peritoneal cavity is filled with high-osmolality glucose solution. The glucose levels in dialysis solution are at least 10 times higher than the level of blood glucose (1500 – 4000 mg/dL). Elevated glucose levels have been reported to stimulate the production of angiotensin II by rat mesangial cells in culture, leading to reduced matrix degradation and accumulation of matrix proteins. Inhibition of the renin–angiotensin system is well known to reduce the progression of renal deterioration in diabetic patients.

Previously, we demonstrated that ARBs protected against loss of residual renal function during CAPD (21). However, no publications have reported on the effect of ARBs on progression of peritoneal fibrosis in patients on CAPD. In the present study, we demonstrated that early intervention with an ARB could protect against the deterioration of peritoneal function and development of EPS sometimes seen on peritoneal dialysis. Based on our results, ARBs are suitable drugs for patients on CAPD, helping to prevent the progression of peritoneal fibrosis.
CONCLUSIONS

Long-term intraperitoneal injection of acidic dialysis solution in rats produced features typical of EPS in humans. In the present study, we demonstrated that early intervention with an ARB could protect against the deterioration of peritoneal function, development of fibrosis, and development of EPS during peritoneal dialysis. By contrast, calcium channel blockers could not provide the same protection. In patients undergoing peritoneal dialysis, ARBs should be the drug of choice because of their effects in reducing the risk of peritoneal fibrosis and EPS.

REFERENCES