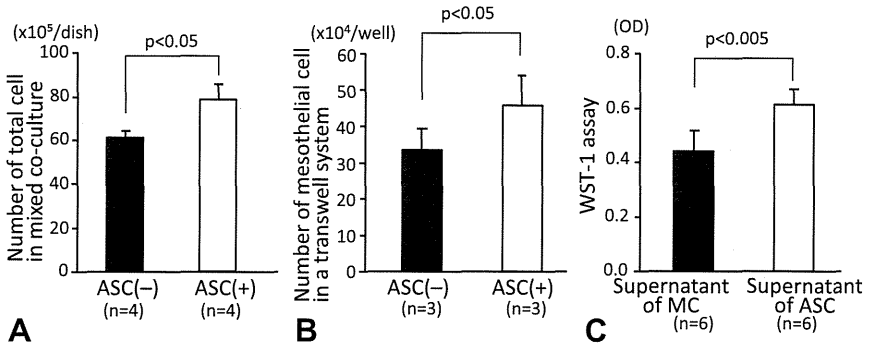

Figure 6. Rat ASCs enhance mesothelial proliferation under co-culture assays. (A) Total number of mesothelial cells mixed with rat ASCs as a co-culture assay. (B) Number of mesothelial cells in lower chamber separated from upper chamber without rat ASCs (ASC(−)) or with rat ASCs (ASC(+)) using the Transwell system. (C) Results of WST-1 cell proliferation assay. Incubation with supernatant from rat ASC culture (supernatant of ASC) shows higher OD values than for mesothelial cells incubated with supernatant from mesothelial cell culture (supernatant of MC).

wounded space of mesothelial monolayers was significantly accelerated by incubation with supernatant from rat ASCs culture (supernatant of ASC in Figure 7) compared with supernatant from mesothelial cell culture (supernatant of MC in Figure 7) at 6 h and 12 h.

Release of HGF was observed from rat ASCs, and proliferation of rat peritoneal mesothelial cells was increased by recombinant rat HGF

When we measured HGF levels in supernatant from rat ASCs, we confirmed the elevation of HGF levels dependent on the initial cell amount of rat ASCs (data not shown) as previously reported (26). To confirm whether HGF released from rat ASCs could directly accelerate proliferation of mesothelial cells in rat peritoneum, we stimulated rat mesothelial cells in medium mixed with various amounts of recombinant rat HGF. Proliferation of rat mesothelial cells increased with increasing amounts of recombinant rat HGF (Figure 8).

Effects of treatment of rat ASCs on day 1 after induction of Zy/scraping peritonitis

When administration of rat ASCs was started on day 1 after induction of Zy/scraping peritonitis (post-ASC(+)), peritoneal thickness was slightly, but not significantly, suppressed compared with lack of treatment of rat ASCs (ASC(−)) (see supplementary Figure 2B). Decreases of total number of infiltration cells and number of ED-1 positive cells were significantly observed in post-ASC(+) rats compared with control rats (ASC(−)) (see supplementary Figure 2C–E).

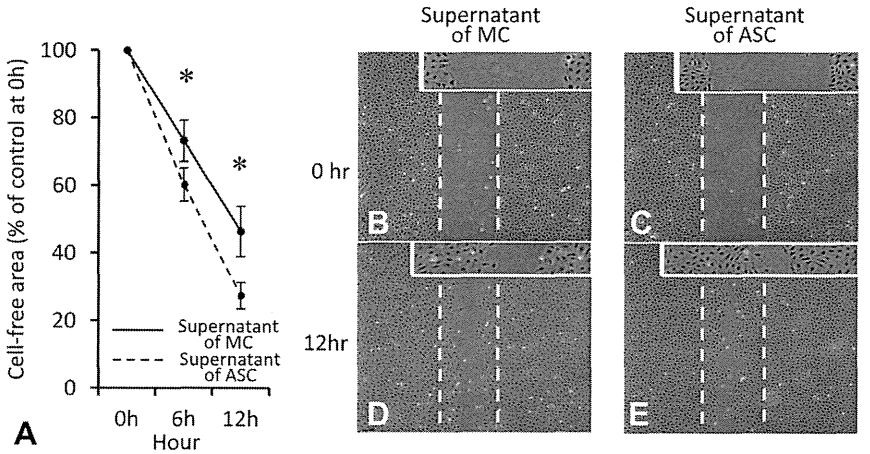

Figure 7. Restoration of injured mesothelial cell monolayers is accelerated by supernatant from rat ASCs in the wound-healing assay. Injured mesothelial cell monolayers were incubated with supernatant of rat ASCs (supernatant of ASC) or of mesothelial cells (supernatant of MC). Percentage of cell-free area at indicated time points compared with percentage of cell-free area at 0 h was determined (A). Each assay was performed in triplicate, and each value represents mean ± SD. Experiments were repeated three times, and results from a representative experiment are shown (*$P < 0.05$). Confluent mesothelial cell monolayers were injured with a pipette tip and treated with supernatant from supernatant of MC (B, D) or from supernatant of ASC (C, E) for the indicated time. Initial injuries are indicated by white dot lines. Cells growing into lines are considered to represent injury closure.

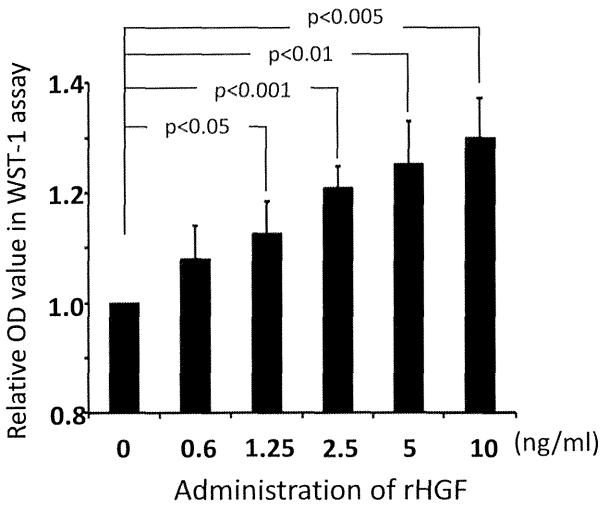

Figure 8. Administration of recombinant rat HGF (rHGF) increased proliferation of mesothelial cells. Each data point represents a relative OD value compared with conditional culture medium without recombinant rat HGF in the WST-1 assay.

Discussion

Rat ASCs have improved injuries from various tissues in more recent reports, including our own reports. Administration of rat ASCs clearly improved urethral resistance (32) and attenuated antibody-mediated rejection (26) and acute kidney injury induced by folic acid (28). In the present study, intraperitoneal injection of rat ASCs significantly suppressed peritoneal injuries in a rat Zy/scraping peritonitis model accompanied by decreased depositions of C3b and C5b–9 as complement activation products. Our results suggest that improvement of peritoneal injuries in rats with rat ASCs was at least partly attributable to suppression of complement activation in peritoneum.

We also investigated the reasons why complement activation and peritoneal injuries could be suppressed by administration of rat ASCs in rat Zy/scraping peritonitis. Although severe peritoneal inflammation developed with defects of the mesothelial cell layer in rats without rat ASC treatments, restoration of the mesothelial cell layer and decreased accumulation of inflammatory cells were observed in the peritoneum of rats treated with rat ASCs. Because mesothelial cells express abundant CRegs such as Crry, CD55 and CD59 (9), recovered mesothelial cells with CRegs in peritoneum might prevent the peritoneum from excessive complement activation in Zy/scraping peritonitis. We also observed that rat ASCs augmented proliferation of mesothelial cells in *in vitro* co-culture assays, and we observed that intraperitoneally injected rat ASCs distributed close to resident/recovered mesothelial cells, supporting the concept that injected rat ASCs facilitated the recovery of mesothelium. Results from our Transwell study suggested that paracrine effects of some factors released from rat ASCs could enhance the restoration of mesothelial cells. Concerning factors released to proliferate mesothelial cells as paracrine effects from rat ASCs, we focused on the roles of HGF for proliferation of mesothelial cells in *in vitro* assays because HGF is reportedly associated with mesothelial proliferation in peritoneal tissue repair (33,34). From our results, HGF released from rat ASCs might help to increase mesothelial cells, which have abundant CRegs. In addition, rat ASCs themselves might have the potential to prevent excessive complement activation locally because rat ASCs also express CRegs (data not shown). As other possibilities for effects of rat ASCs among mesenchymal stromal cells acting to protect against peritoneal injures, HGF released from mesenchymal stromal cells might decrease peritoneal tissue injures through anti-apoptotic effects (35). Another possibility involves factor H, a complement regulator, which is reportedly released from human mesenchymal stromal cells, suggesting that mesenchymal stromal cells themselves might have potential direct effects in protecting against complement activation (36).

Usage of ASCs in particular may prove advantageous because ASCs are easily and safely harvested from abundant adipose tissues compared with bone marrow-derived stromal cells. As mesenchymal stromal cells, ASCs may have multiple applications for tissue engineering and improvement of tissue injuries (37,38). As one aspect of the effects of ASCs, autocrine and paracrine effects of ASCs have been reported, such as regeneration of injured cardiac tissue using stem cell-derived cardiomyocyte sheets in a swine model of chronic myocardial infarction (39). Clinically, some trials of mesenchymal stromal cells have already succeeded in improving acute rejection of renal transplantation (40) and reducing infarction size in ischemic cardiomyopathy (41). In contrast, as unexpected problems, ASCs might present similar problems to the therapeutic use of mesenchymal stromal cells, such as infections, carcinogenesis, promotion of cancer metastasis or allergy caused by contaminating proteins such as growth factors (42–44). Although ASCs work to host with autocrine and paracrine effects, the present results suggest that paracrine effects of rat ASCs might play protective roles against peritoneal injuries.

In conclusion, our results suggest that rat ASCs facilitated recovery of mesothelial cells in the peritoneum, partly through suppression of complement activation, although how rat ASCs improve peritoneal injuries in Zy/scraping peritonitis remains unclear. In our study, administration of rat ASCs after

induction of Zy/scraping peritonitis limited the peritoneal injuries because the induced peritoneal injuries were severe. The present results suggest that administration of rat ASCs might provide a therapeutic approach to regulate the complement activation system in injured peritoneum under conditions of yeast-related peritonitis.

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Supplementary data

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ORIGINAL ARTICLE

Artificial Kidney / Dialysis

Morphological characteristics in peritoneum in patients with neutral peritoneal dialysis solution

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Abstract Peritoneal dialysis solution (PDS) plays a role in functional and morphological damage to the peritoneum. This study aimed to clarify the effect of neutral PDS in preventing morphological changes by assessing peritoneal damage and comparing morphological alterations between PD patients treated with neutral PDS and acidic PDS. Sixty-one patients participated from seven hospitals. All patients were treated with neutral PDS excluding icodextrin, during their entire PD treatment, and experienced no episode of peritonitis. The thickness of submesothelial compact (SMC) zone and the presence of vasculopathy in the anterior parietal abdominal peritoneum were assessed. The impact of icodextrin, hybrid therapy, and peritoneal rest and lavage in morphological alterations were determined. There was no significant difference in the average SMC thickness between neutral and acidic PDS. The vessel patency in patients using neutral PDS was significantly higher compared to that in acidic PDS at any time during PD. There were no significant suppressive effects from interventions or use of icodextrin with respect to peritoneal morphological injury. A monolayer of mesothelial cell was observed in approximately half the patients, especially in their receiving lavage patients. Neutral PDS, accompanied by other preventive approaches against peritoneal injury, might suppress the development of peritoneal morphological alterations.

Keywords Peritoneal dialysis · Neutral PD solution · Pathological changes · Hybrid therapy · Peritoneal lavage

On behalf of the Peritoneal Biopsy Study Group of the Japanese Society for Peritoneal Dialysis.

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Introduction

Peritoneal dialysis (PD) has been an important renal replacement therapy over past several decades. Conventional PD solution is potentially bioincompatible because of its hyperosmolarity, low pH, and high lactate and glucose concentration [1, 2]. Glucose in conventional PD solution is affected by heat sterilization and converted to the substrate for glucose degradation products (GDPs), such as methylglyoxal, formaldehyde, 3-deoxyglycosone, acetaldehyde, 2-furaldehyde and 5-hydroxymethyl furaldehyde [3, 4]. Furthermore, glucose in PD solution is involved in providing the substrate for nonenzymatic glycation of tissue protein, which is a substance detrimental to peritoneum [5]. Although the acidity of conventional PD solution plays a role of preventing bacteria from growing during storage and glucose degeneration by heat sterilization, acidity causes dysfunction of mesothelial cells and peritoneal fibroblasts, as well as impairment of immune defense in the peritoneal cavity through deteriorating intracellular pH [4, 6]. Additionally, long-term exposure to bioincompatible PD solution results in various functional and morphological alterations in the peritoneum of PD patients.

Morphological characteristics of the peritoneum in PD patient who use bioincompatible PD solution include loss of mesothelial cells, thickening of the peritoneal interstitium, and vasculopathy in small vessels [2, 7]. Ultrafiltration failure associated with increased peritoneal permeability and impairment of immune defense in peritoneal cavity are characteristics of the major functional abnormalities induced by the bioincompatible PD solution [8]. Recently, greater biocompatibility of PD solution has been achieved through a more physiological pH, lower osmolarity, a decreased glucose load, and lower GDP content compared with the conventional acidic PD solutions [9]. A new biocompatible PD solution has been reported to provide some clinical benefits [10]. A higher concentration of cancer antigen (CA)-125 and a lower concentration of hyaluronan in overnight PD effluent have been observed in patients treated with the new biocompatible solution, indicating less mesothelial and interstitial damage [9, 10]. Several new strategies, such as using biocompatible PD solution in combination with peritoneal rest, peritoneal lavage and hemodialysis, have been implemented to prevent and reduce peritoneal injuries. These new strategies are expected to have a positive impact on the clinical and morphological alterations in the PD peritoneum.

The purpose of the present study was to examine the effects of neutral PD solution and several interventions with regard to PD treatment on morphological alterations in PD patients.

Materials and methods

Patients

Peritoneal biopsy specimens were obtained from 61 patients, who were treated with neutral peritoneal dialysate alone during PD therapy. The biopsies were performed during surgery for PD catheter removal prior to transfer to hemodialysis ($n = 60$), and during surgery for non PD-related abdominal disease ($n = 1$). Patients with history of peritonitis during PD therapy were excluded from this study; therefore, any influences by peritonitis on a study patient's peritoneal histology were eliminated in this study. The existence of diabetes was identified from clinical records. Approval for the study was obtained from the local ethics committees, and all patients gave written informed consent.

Peritoneal rest is the drainage of peritoneal effluent every day or every other day in PD patients transferred from PD to hemodialysis and scheduled to undergo PD catheter removal several months after PD cessation. Peritoneal lavage is lavage of the peritoneal cavity with PD solution every day or every other day in PD patients transferred to hemodialysis and scheduled to undergo PD catheter removal several months after PD cessation. The studied PD patients, who were experiencing dialysis that was nearly inadequate or overhydration caused by deterioration of residual renal function, had been undergoing hemodialysis once a week additionally as standard hybrid therapy.

Peritoneal biopsy samples ($n = 80$) were taken from PD patients who were treated with acidic peritoneal dialysate as control group, these were the same specimens used in our previous study [9]. The patients, whose average PD duration was 62.5 ± 43.3 months, had no history of peritonitis during their PD therapy.

Processing of biopsy samples

Forty-eight specimens out of the 61 participated patients were sampled near the site of catheter insertion during the routine surgical procedure of PD catheter removal. In 11 patients who underwent endoscopic surgical procedure and two patients who underwent open abdominal surgery, parietal peritoneal specimens from the anterior abdominal wall were obtained at the opposite site of catheter insertion.

Samples of the parietal peritoneum were biopsied in the usual manner. Briefly, the peritoneal tissue was cut by scalpel measuring approximately 1 cm in size and up to 5 mm in depth. Each tissue sample was placed on a small board or filter paper with the mesothelial surface uppermost, been extended to the same size as in situ, and fixed with 20 % buffered formalin. After overnight fixation at

room temperature, the samples were routinely processed for light microscopy and embedded in paraffin. The 4-μm sections were cut routinely and stained with hematoxylin and eosin and Masson trichrome.

Sample analysis methods

The samples were assessed by microscopy using a standardized method described next. Two experienced examiners, one pathologist (K. H.) and one nephrologist (C. H.), who were unaware of patients' clinical backgrounds, evaluated the samples independently.

Adequacy of specimen for histologic evaluation

The adequacy of each specimen for histologic evaluation of peritoneal thickness and vasculopathy was determined independently. For measurement of peritoneal thickness and vasculopathy, each specimen was assessed in terms of size, site, and direction of the specimen, and classified as an "adequate" or "inadequate specimen". An adequate specimen had a sampling size that was large enough and contained several layers of peritoneum (mesothelial, submesothelial, and adipose tissue layers). The direction of embedding was almost vertical so that the thickness of the submesothelial layer could be measured properly. For the evaluation of vasculopathy, an adequate specimen must contain a post-capillary venule (PCV) at the size of 25–50 μm in external diameter. The number of adequate and inadequate specimens for the evaluation of peritoneal thickness was 30 and 31, respectively, and for the evaluation of vasculopathy was 39 and 22, respectively. In patients using acidic PD solution, 40 samples from the 80 PD patients were adequate for the evaluation of peritoneal thickness and 76 samples from the 80 PD patients were adequate for the evaluation of vasculopathy.

Evaluation of peritoneal fibrosis

The extent of peritoneal fibrosis was determined by the thickness of submesothelial interstitial layer (submesothelial compact zone: SMC) between the basal border of the surface mesothelial cells and the upper border of the peritoneal adipose tissue. The thickness of the mesothelial cell layer was excluded from measurement when it was present on the peritoneal surface. When the submesothelial interstitium was continuous to underlying dense connective tissue (abdominal fascia) without peritoneal adipose tissue, the peritoneal thickness could not be measured. Five portions were randomly selected for the measurement of submesothelial (SMC) thickness (Fig. 1a). The thickness was measured by a micrometer on a microscopic lens or by an image analyzer, and then the average SMC thickness was calculated. The portion where the peritoneum appeared severely fibrotic as a result of tangential embedding or miscellaneous inflammatory reactions was excluded from measurement.

Evaluation of vasculopathy

The extent of vasculopathy was determined by the severity of luminal narrowing at the level of the PCVs. For evaluation of the severity of luminal narrowing, the ratio of luminal diameter to vessel external diameter (*L/V*) was determined (Fig. 1b), which represents the extent of patency of a blood vessel, according to our previous report [7]. In general, hyalinizing vasculopathy in PD patients is usually observed at the PCV or capillary level. For morphologic measurement, we selected a PCV whose diameter ranged from 25 to 50 μm, since the *L/V* was influenced by the level of blood vessel examined [11]. The measurement was obtained on the short axis to avoid the artificial effect of elongated distance as a result of tangential cutting of the

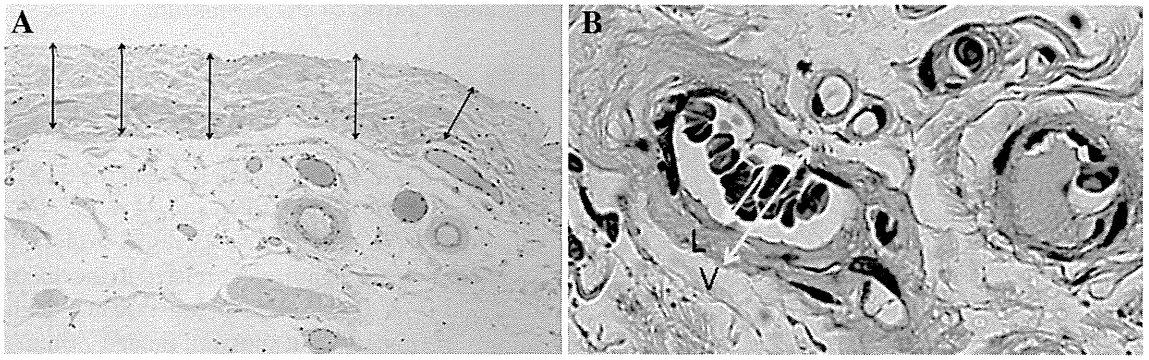

Fig. 1 **a** For the evaluation of peritoneal fibrosis, the peritoneal thicknesses at randomly selected five points were measured, and then the average peritoneal thickness (APT) was calculated. The average of two APT values determined by two examiners was taken as a representative APT of that case. **b** For the evaluation of vascular patency, the average ratio of lumen-to-vessel (*L/V*) diameter was calculated at five randomly selected post-capillary venules (PCVs) with external diameters of 25–50 μm

vessel during histologic preparation. When different vessels showed different severities of vasculopathy, five vessels of each specimen were chosen for measurement. The average of two thicknesses of vascular wall and *L/V* measured by two examiners was taken as the representative value of that case.

Evaluation of surface coverage with mesothelial cells

Forty-three out of 61 specimens were assessable for surface coverage. For semi-quantitative assessment, we classified four grades according to the coverage with mesothelial cells: grade 0, none: 1, <25 %; 2, 25–50 %; 3, >50 %.

Statistical analyses

Data are expressed as mean ± SD. Parametric comparison was performed by Student's *t* test, and nonparametric comparison was conducted by the Mann–Whitney *U* test to examine statistical significance. Relationships between clinical variables and morphological alterations were analyzed with Spearman's correlation coefficient. Repeated measures analysis of variance (ANOVA) between morphologic changes and the interventions was performed. Multivariate analysis of variance and covariance was used to assess the impact of clinical parameters and interventions on morphological changes. Statistical analysis was performed with StatView version 5.0 software. $p < 0.05$ was considered to be significant.

Table 1 Characteristics of participants

Age (years)	53.1 ± 15.1 (2.4–81)
Gender	
Male: female	42: 19
Causes of CKD (patients)	
DM	19
Non-DM	39
Unknown	3
Duration of PD (months)	37.0 ± 24.9 (2.9–117.2)
Prescription of PD	
CAPD	57
APD	4
Daily UV (patients)	
≥200 mL	18
<200 mL	27
Unknown	16
Use of 2.5 % glucose PDS (patients)	36
Use of 42.5 % glucose PDS (patients)	0
Icodextrin (patients)	22
ARB/ACEI (patients)	38/1
HT Tx unknown (patients)	16
Hybrid Tx (patients)	15
Peritoneal rest (patients)	18
Peritoneal lavage (patients)	27

CKD chronic kidney disease, *UV* urinary volume, *PDS* peritoneal dialysis solution, *ARB* angiotensin receptor blocker, *ACEI* angiotensin-converting enzyme inhibitor, *HT* hypertension, *Tx* treatment, *DM* diabetes mellitus, *CAPD* continuous ambulatory peritoneal dialysis, *APD* automated peritoneal dialysis

Results

Clinical background of participants

The clinical background of the participants is shown in Table 1. Fifty-seven patients were undergoing continuous ambulatory peritoneal dialysis (CAPD) and four patients were on automated peritoneal dialysis (APD). The average PD duration was 33.6 ± 23.1 months. Most of the participants underwent PD catheter removal because of planned transfer from PD to hemodialysis. Among the 61 PD patients, four were regarded as having impaired ultrafiltration capacity (UFC), which was defined by use of more than four hypertonic bags (2.5 % glucose PD solution or icodextrin) in each 24 h period to maintain their solution balance. No PD patient used 4.25 % PD solution in this study. Twenty-seven patients had less than 200 mL urinary volume per day. Fifteen patients received hybrid therapy to manage body fluid balance or a uremic condition. Peritoneal rest and lavage were performed in 18 and 27 patients, respectively, after transfer to hemodialysis simultaneously. As control group, 80 patients using acidic PD solution participated. As for the cause of chronic kidney disease (CKD), 45 patients were with chronic glomerulonephritis (CGN), 9 patients with diabetes (DM), and remaining 26 with other disease or unknown. The average age was 47.3 ± 15.5 years, and the duration of PD was 62.5 ± 43.3 months.

Peritoneal fibrosis of PD peritoneum

In the studied PD patient using neutral PD solution alone, the average peritoneal thickness was 296.7 ± 132.5 μm ranging from 93.2 to 722.6 μm. The sampling site from the PD catheter insertion did not aggravate the SMC thickness (the near site, 287.8 ± 136.5 μm, $n = 22$; the opposite site, 376.7 ± 40.9 μm, $n = 9$, respectively). Peritoneal thickness was not significantly related to the duration of PD treatment (Fig. 2a). In the PD patient using acidic PD solution, peritoneal thicknesses ranged from 45.5 to 777.9 μm. The average SMC thickness was 266.2 ± 159.9 μm ($n = 40$). The SMC thickness significantly increased with PD duration (Fig. 2b). There was no significant difference in SMC between patients using

neutral PD solution and using acidic PD solution for at least 60 months (Fig. 2c).

Peritoneal vasculopathy of PD peritoneum

In PD patients using neutral PD solution, the average *L/V* at PCV was 0.801 ± 0.075, ranging from 0.596 to 0.906 ($n = 39$). The sampling site from the PD catheter insertion was not aggravated by vascular stenosis (the near site, 0.80 ± 0.08, $n = 15$; the opposite site, 0.80 ± 0.12, $n = 7$). The *L/V* decreased with the PD duration in the PD patients using neutral PD solution (Fig. 3a). The *L/V* in the PD patients using acidic PD solution also decreased with PD duration (Fig. 3b). The *L/V* ranged from 0 to 0.869, and the average *L/V* at PCV was 0.494 ± 0.296 in PD patients using acidic PD solution ($n = 66$). The attenuation slope of *L/V* in the acidic PD solution was greater compared with that in the neutral PD solution (Fig. 3b). A decrease in *L/V* of the patient who used acid PD solution for at least 60 months was markedly rapid compare to that of the patients with neutral PD solution (Fig. 3c).

Effect of neutral PD solution on surface coverage with mesothelial cells

Mesothelial cells were presented in 24 out of 43 specimens. The duration of PD was no significantly different between grade 0 and grade 1, 2 and 3 (38.2 ± 21.5 and 33.7 ± 30.4 months, respectively). Mesothelial cells were found in four patients who had undergone PD for more than 5 years. Cubical mesothelial cells was found in 22 specimens, and stratified mesothelium was found in 16 patients.

Impacts of interventions for peritoneal morphologic changes

Sixty-one patients whose specimens were evaluable were divided into four groups according to interventions such as

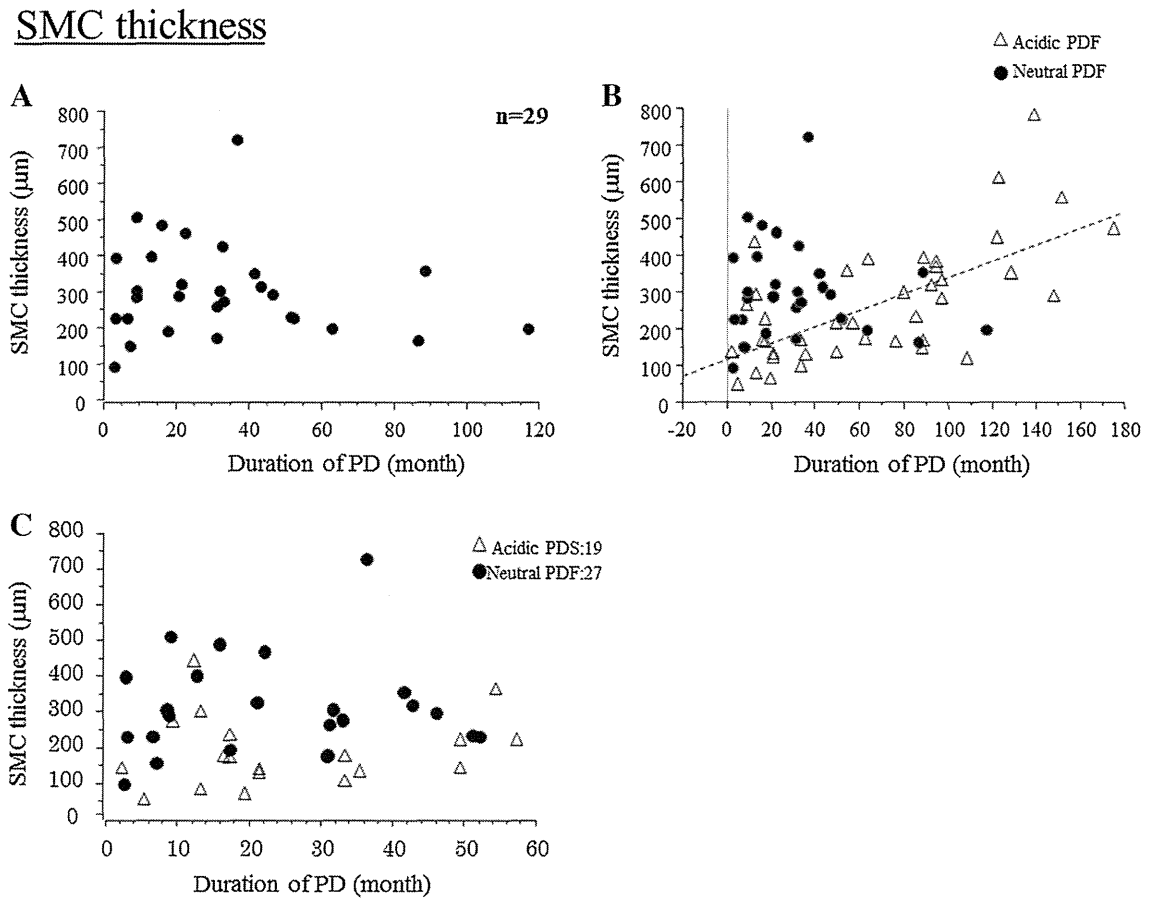

Fig. 2 Average peritoneal thickness (APT) by five-point measurement method. The peritoneal thicknesses at randomly selected five points were measured, and then the APT was calculated (**a**). The relationship between SMC and PD duration in patients using neutral and acidic PD solution (**b**). The relationship between SMC and PD duration in patients using neutral PD solution and patients using acidic PD solution for at least 60 months (**c**). Patients using acidic PD solution show in *open triangle*. Patients using neutral PD solution show in *filled circle*

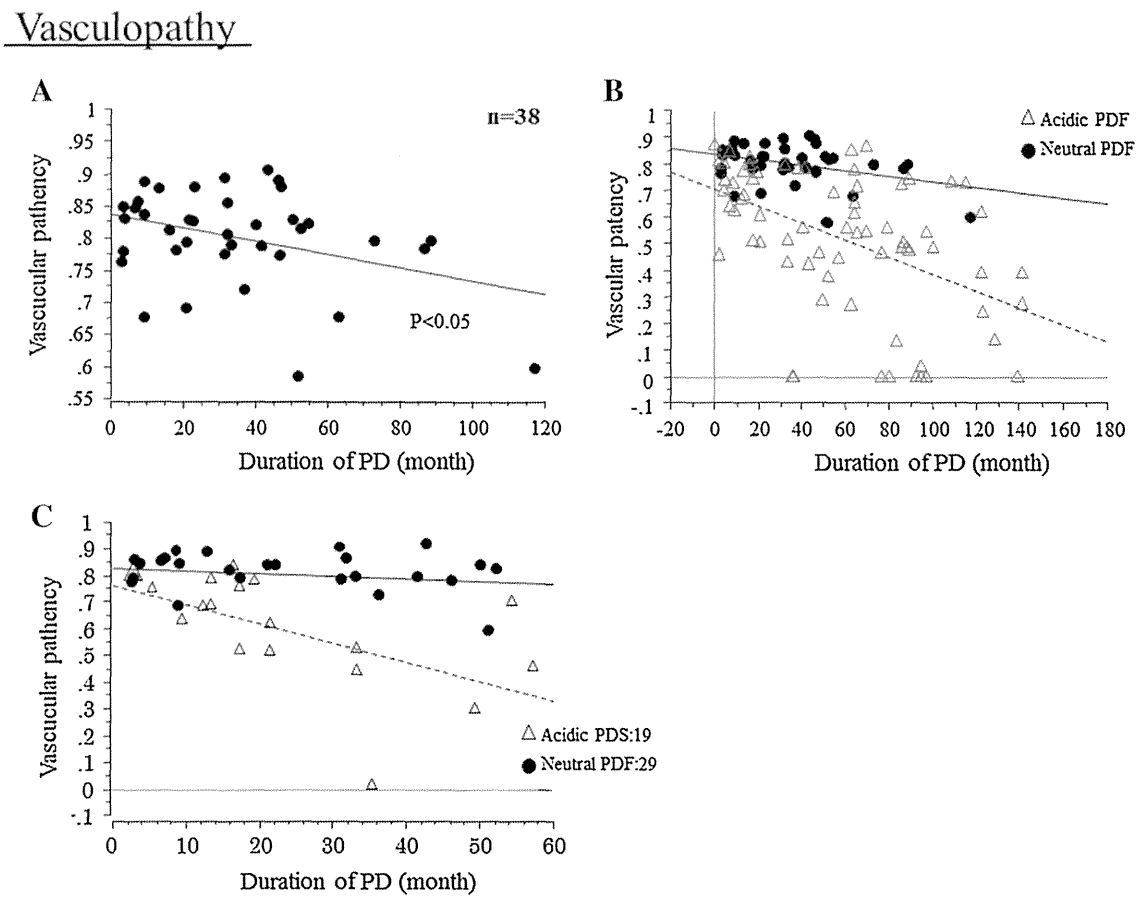

Fig. 3 Quantitative evaluation of vasculopathy at post-capillary venules (PCVs). For evaluation of the severity of luminal narrowing, the ratio of lumen-to- vessel diameter (*L/V*) was determined, representing the extent of patency of the blood vessel. The *L/V* decreased with PD duration in patients using neutral PD solution (**a**). The relationship between *L/V* and PD duration in patients using neutral and acidic PD solution (**b**). The relationship between *L/V* and PD duration in patients using acidic PD solution for at least 60 months (**c**). Patients using acidic PD solution show in *open triangle*. Patients using neutral PD solution show in *filled circle*

hybrid therapy and peritoneal rest and lavage (Table 2). The duration of PD in patients who had undergone peritoneal lavage was significantly longer than in patients who had undergone PD alone ($p < 0.05$). The degree of surface coverage with mesothelial cells in the patients who had undergone hybrid therapy was lower than in the patients without hybrid therapy. The use of icodextrin had no influence on the prevention of morphologic changes.

Impact of icodextrin for peritoneal morphologic changes

We analyzed the independent impact of clinical parameters such as PD duration, residual renal function (RRF), icodextrin, usage of 2.5 % glucose PDS, hybrid therapy and peritoneal rest/lavage on peritoneal morphological changes in the multivariate analysis (Table 3). PD duration was an independent coefficient factor for vascular patency.

Discussion

Peritoneal dialysis (PD), one of the established renal replacement treatments worldwide, has advantages over hemodialysis (HD) in the preservation of residual renal function and enablement of high quality of life. The limited timeframe of PD treatment is a matter of concern, since progression of the functional and morphological alterations and increase of encapsulating peritoneal sclerosis (EPS) incidence occur with lengthening PD duration. Bioincompatible PD solution, containing high concentration of glucose and lactate and low pH is a major deteriorating factor of the peritoneal injury that accompanies PD. The conventional acidic PD solution including high concentration of glucose, lactate, and GDP induced an increased state of carbonyl stress on the peritoneum and promoted the development of peritoneal sclerosis [12, 13].

Table 2 Impacts of interventions in peritoneal morphological changes

	Hybrid therapy		PD alone	
	Peritoneal lavage/rest ($n = 10$)	None ($n = 5$)	Peritoneal lavage/rest ($n = 11$)	None ($n = 14$)
Duration of PD (month)	44.2 ± 20.4*	34.9 ± 21.6*	43.5 ± 34.4*	19.1 ± 16.9
SMC thickness (μm)	233.8 ± 72.7	330.0 ± 227.3	302.1 ± 90.2	306.0 ± 141.9
Vascular patency	0.80 ± 0.10	0.76 ± 0.07	0.79 ± 0.09	0.82 ± 0.04
Grade of mesothelial preservation	0.3 ± 0.5#	0.8 ± 0.5	1.8 ± 1.3	1.3 ± 1.2

* $p < 0.05$, vs PD alone, # $p < 0.02$ vs peritoneal lavage/rest alone

UV urinary volume, *PDS* peritoneal dialysis solution, *SMC* submesothelial compact zone

Table 3 Multivariable analysis of selected clinical parameters for pathological changes

Variables	Coefficient	CI	*p* value
SMC thickness			
Grade of RRF	−23.8	−301.6 to 254.0	0.851
Vascular patency			
PD duration	−0.001	−0.002 to 0.000	0.027
Grade of RRF	0.023	−0.069 to 0.114	0.633
Hybrid therapy	−0.041	−0.128 to 0.046	0.411
Icodextrin	0.007	−0.095 to 0.108	0.892
Time of 2.5 % PDS	0.006	−0.038 to 0.049	0.787

SMC submesothelial compact zone, *CI* confidential interval, *RRF* residual renal function, *PDS* peritoneal dialysis solution

Recently, peritoneal morphologic changes in patients using neutral PD solution have been reported by Ayuzawa and Kawanishi [14, 15]. However, these authors indicated that the neutral PD solution was able to minimize the functional and morphological peritoneal damage compared with the amount of damage reported in previous studies. In present study, we compared the impact of acidic and neutral PD solution on peritoneal morphological changes using quantitative assessment in a multicenter study. The usefulness of neutral PD solution in preventing peritoneal morphological injury was clarified, especially pertaining to vasculopathy in PD patients.

Peritoneal morphological alterations in PD patients in the era of acidic PD solution are characterized by mesothelial injury, SMC thickening and vasculopathy in small vessels. Flat mesothelial cells are transformed into cubical cells, and then detached from the peritoneal surface. Vascular stenosis and obliteration caused by hyalinized thickening of vascular walls in PCVs and capillaries and angiogenesis are found as vasculopathy in PD patients. Finally long exposure to PD solution results to be the denudate thickens peritoneum [2, 7]. Willians [2] and Honda [7] reported that SMC thickness progressively increased with PD duration. In particular, vascular patency decreased with increasing PD duration [7, 11]. Mateijsen [16] examined the relationship between morphological changes and PD duration in 15 uremic patients and 25 PD patients including 11 peritoneal sclerosis patients. They revealed that neoangiogenesis and vasodilatation of the capillaries can be found in long-term CAPD patients. Participants were transferred to hemodialysis by planned discontinuation according to the guidelines for peritoneal dialysis (PD) of the Japanese Society for Dialysis Treatment 2009 [17] excluding one patient with ultrafiltration failure. The SMC thickness increased with the use of neutral PD solution (average SMC thickness; 296.7 ± 132.5 μm), however, there was no relationship between the SMC thickness and PD duration in the present study. Vascular patency decreased with lengthening PD duration in this observation. Conventional acidic PD solution strongly accelerated the progression of vasculopathy compared with neutral PD solution. Vascular patency in PCVs is related to peritoneal permeability and the dialysate-to-plasma ratio of creatinine (D/P creatinine) in the peritoneal equilibration test (PET) [11]. Previous neutral PD fluid study [9] indicated that the use of a neutral pH, low GDP fluid, is accompanied by a significant improvement in effluent markers of peritoneal membrane integrity. Therefore, the improvement in ultrafiltration and peritoneal transport might be ascribed to the sustained vascular patency in patients receiving neutral PD solution.

Half of the participants were not administered hyperosmotic PD solution. Twenty-four out of 57 patients received icodextrin for adequate ultrafiltration. Additionally, hybrid therapy, peritoneal rest, and lavage were performed in many participants as strategies of preventing peritoneal injury. The reasons of these interventions were variable depending on the individual patients' clinical backgrounds and their physicians' opinions concerning dialysis modality. The duration of PD was not different between the patients who did and did not receive interventions. There were no differences in SMC and vascular patency between the patients with and without intervention. The possibility that peritoneal lavage promoted a cover by mesothelial

cells was suggested in this study. Previous studies [9, 10] reported the elevation of Cancer Antigen 125 (CA-125) in the effluent of patients using neutral PD solution. In a cohort study of 247 PD patients, Yamamoto [18] pointed out that the overall incident of EPS was significantly lower in the lavage group than in the non-lavage group. Since mesothelial cells play a central role in the peritoneal repair after acute injury, such as bacterial infection or operative procedures, the preservation of mesothelial cells might be related to the peritoneal repair after PD withdrawal. Therefore, the use of neutral PD solution together with combined interventions can play a favorable role on preventing or improving peritoneal morphological alterations. An additional large clinical study is needed to establish a consensus on the usefulness of the different interventions in PD patients.

The peritoneal specimens in PD patients using acidic PDS, obtained for assessment of basic morphologic findings in Japan, were utilized as control [7]. According to the small number of participants and lack of baseline clinical data in the participants using acidic PDS, we could not provide the relationship between the morphological and the functional improvement and the impact of peritoneal lavage and rest, hybrid therapy and use of icodextrin on peritoneal injury in this study. Only neutral PDS has been available in Japan since 2004. Further studies are needed to determine whether these beneficial impacts of the neutral PD solution could improve the long-term clinical outcome of the PD patients.

In conclusions, neutral PD solution is more biocompatible with respect to preventing morphological injury than acidic PD solution for PD.

Conflict of interest The authors have no conflicts of interest to declare expect Dr. Yasuhiko Ito. He is a professor in endowed chair supported by Baxter Japan.

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Long-Term Effects of Spironolactone in Peritoneal Dialysis Patients

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ABSTRACT

ESRD treated with dialysis is associated with increased left ventricular hypertrophy, which, in turn, is related to high mortality. Mineralocorticoid receptor antagonists improve survival in patients with chronic heart failure; however, the effects in patients undergoing dialysis remain uncertain. We conducted a multicenter, open-label, prospective, randomized trial with 158 patients receiving angiotensin-converting enzyme inhibitor or angiotensin type 1 receptor antagonist and undergoing peritoneal dialysis with and without (control group) spironolactone for 2 years. As a primary endpoint, rate of change in left ventricular mass index assessed by echocardiography improved significantly at 6 (P=0.03), 18 (P=0.004), and 24 (P=0.01) months in patients taking spironolactone compared with the control group. Rate of change in left ventricular ejection fraction improved significantly at 24 weeks with spironolactone compared with nontreatment (P=0.02). The benefits of spironolactone were clear in patients with reduced residual renal function. As secondary endpoints, renal Kt/V and dialysate-to-plasma creatinine ratio did not differ significantly between groups during the observation period. No serious adverse effects, such as hyperkalemia, occurred. In this trial, spironolactone prevented cardiac hypertrophy and decreases in left ventricular ejection fraction in patients undergoing peritoneal dialysis, without significant adverse effects. Further studies, including those to determine relative effectiveness in women and men and to evaluate additional secondary endpoints, should confirm these data in a larger cohort.

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ESRD treated by hemodialysis or peritoneal dialysis (PD) is associated with a high prevalence of increased left ventricular (LV) mass (left ventricular hypertrophy [LVH]), fibrosis, and capillary loss.[1,2] CKD exposes the heart to three major mechanisms that facilitate the development of cardiomyopathy and induce LV failure: pressure overload, volume overload, and CKD-related nonhemodynamic factors that alter the myocardium.[3] The persistence and severity of LVH are closely associated with cardiovascular and all-cause mortality.[3–6] Terminal events in LVH are known to include pump failure and sudden arrhythmic death.[3,7–9] Furthermore, ischemic disease, as exemplified by coronary atherosclerosis, is a less important factor in cardiovascular mortality and morbidity from CKD and ESRD.[1] Therefore, a new paradigm of therapy for ESRD that prioritizes prevention and reversal of LVH and cardiac fibrosis is needed.[1]

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Mineralocorticoid receptor (MR) antagonists are reported to improve the survival of patients with chronic heart failure and heart failure that occurs after myocardial infarction[10–12] and to reduce LV mass;[13] however, in patients undergoing dialysis, the few reports on the effects of MR blockade have typically focused on small cohorts that were underpowered to allow conclusions to be drawn.[14–17] We explored the effects of concurrent spironolactone and angiotensin-converting enzyme inhibitor (ACEI) or angiotensin type 1 receptor blocker (ARB) on cardiac complications (LV mass index [LVMI] and LV ejection fraction [LVEF]), peritoneal membrane, and residual renal functions in a multicenter, open-label, prospective controlled trial of patients undergoing PD.

RESULTS

Patient Characteristics at Baseline

A total of 158 patients under treatment with ACEI or ARB and undergoing peritoneal dialysis were randomly assigned to receive spironolactone (n=78) or not receive spironolactone (control group, n=80) (Figure 1). None of the patients were receiving both an ACEI and an ARB at the start of the study. Primary causes of renal failure were as follows: diabetes mellitus (39.2%), chronic GN (31.5%), nephrosclerosis (21.5%), other (3.8%), or unknown (4.4%). Patients were younger than the mean age of 66.2 years seen in Japanese dialysis patients (mean age±SD, 57.4±12.27 and 55.6±14.37 years in the treatment and control groups, respectively).[18] Duration of PD treatment at baseline was relatively short (6 and 7.5 months, respectively), and urine volume was preserved (1000 and 955 ml, respectively). Baseline values for rate of use of icodextrin solution, LVMI, LVEF, BP, dialysate-to-plasma creatinine ratio (D/P Cr) by peritoneal equilibration test, renal Kt/V urea, urine volume, serum brain natriuretic peptide, and aldosterone levels were similar between the groups. Other demographic and baseline characteristics in the randomized population were similar, except for the rate of furosemide use (Table 1).

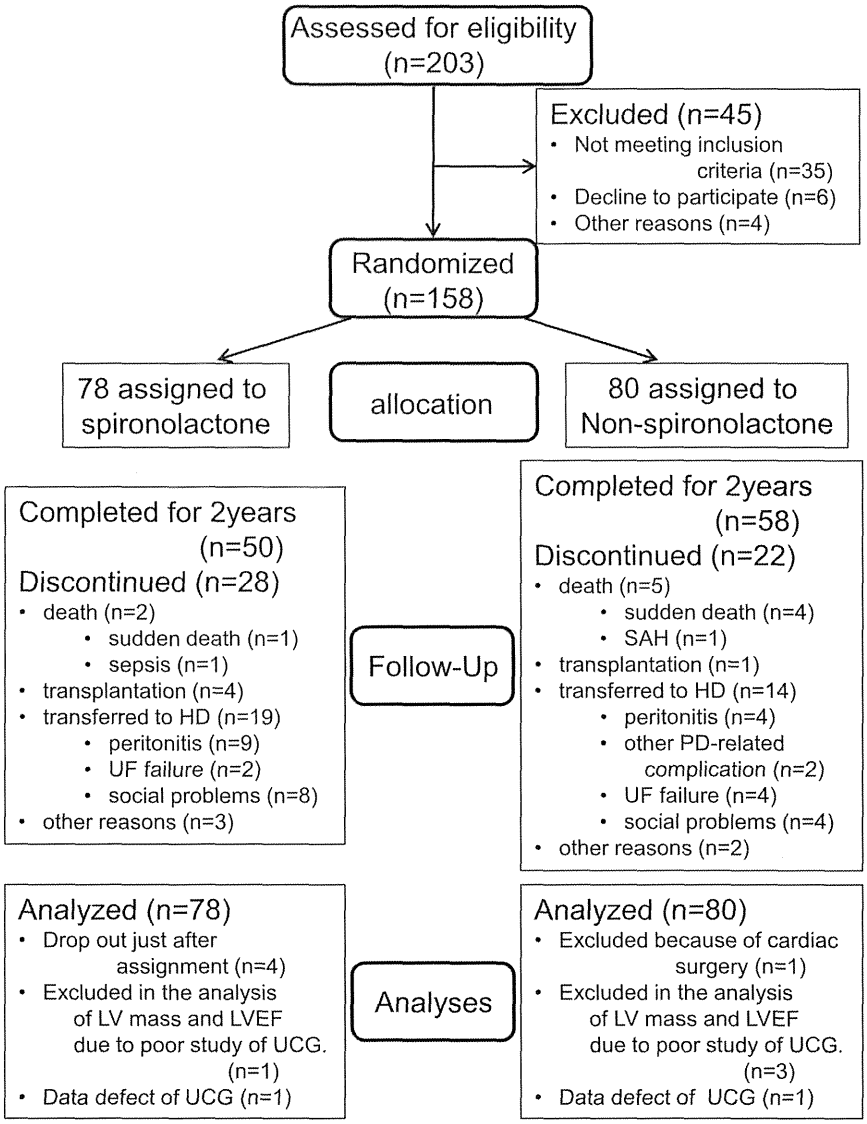

Figure 1. Number of patients enrolled, randomly assigned, and analyzed as part of the study. HD, hemodialysis; SAH, subarachnoid hemorrhage; UCG, echocardiography; UF, ultrafiltration.

Treatment Characteristics

Systolic and diastolic BP pressure did not significantly differ between the groups during the study period (P=0.65 and P=0.76 at 24 months, respectively) (Supplemental Figure 1).

Primary Outcomes

Changes in LVMI

Rate of change in LVMI assessed by echocardiography was significantly improved in the spironolactone group at 6 (P=0.03 versus control), 18 (P=0.004 versus control), and 24 (P=0.01 versus control) months compared with the nontreatment control group (Figure 2A). Because we observed interactions between women and men (P=0.01), we separately analyzed the effects of spironolactone. The effect of spironolactone on rate of changes in LVMI was similar in men with LVMI≤50 g/m$^{2.7}$ (normal range for men) and LVMI>50 g/m$^{2.7}$ (LVH)[19,20] at baseline (P for interaction=0.21). The rate of change in LVMI for men with LVMI≤50 g/m$^{2.7}$ (normal range) at baseline did not change

Table 1. Baseline characteristics of participants

Characteristic	Spironolactone Group (*n*=78)	Control Group (*n*=80)	*P* Value
Age (yr)	57.4±12.27	55.6±14.37	0.40[a]
Women, *n* (%)	23 (29.5)	22 (27.5)	0.86[b]
Diabetes mellitus, *n* (%)	33 (42.3)	29 (36.3)	0.52[b]
Use of icodextrin, *n* (%)	22 (28.2)	23 (28.8)	1[b]
Time receiving PD (mo)	6 (2.0–21.0)	7.5 (2.0–22.5)	0.57[c]
NYHA-FC, *n* (%)			
I	77 (98.7)	80 (100)	0.49[b]
II	1 (1.3)	0 (0)	
Height ($m^{2.7}$)	3.8±0.55	3.8±0.47	0.69[a]
Systolic BP (mmHg)	137.4±15.42	135±16.56	0.16[a]
Diastolic BP (mmHg)	76.3±10.39	78.7±11.60	0.18[a]
Hemoglobin (g/dl)	10.4±1.31	10.3±1.24	0.85[a]
Total protein (g/dl)	6.3±0.64	6.3±0.60	0.66[a]
Serum albumin (g/dl)	3.3±0.51	3.4±0.49	0.36[a]
TC (mg/dl)	184 (162.0–200.0)	182.5 (152.5–209.0)	0.74
LDL-C (mg/dl)	106 (87.0–125.0)	106 (81.5–135.0)	0.59
Na (mEq/L)	137.8±3.65	138.4±3.40	0.33[a]
K (mEq/L)	4.3±0.66	4.3±0.65	0.69[a]
P (mg/dl)	5.4±1.54	5.2±1.28	0.44[a]
Ca (mg/dl)	8.8±0.88	8.7±1.13	0.58[a]
Blood urea (mg/dl)	52.9 (42.9–64.9)	54 (45.7–63.6)	0.58[c]
Serum creatinine (mg/dl)	8.3 (6.6–10.8)	8.5 (6.7–11.2)	0.67[c]
β_2-MG (μg/ml)	20.6±5.66	22.6±8.02	0.08[a]
Urine volume (ml/d)	1000 (500.0–1650.0)	955 (550.0–1400.0)	0.30[c]
Weekly renal Kt/V urea	0.81 (0.41–1.08)	0.66 (0.41–1.06)	0.45[c]
Weekly PD Kt/V urea	1.13 (0.70–1.40)	1.17 (0.75–1.53)	0.39[c]
D/P Cr 4 h	0.66 (0.59–0.75)	0.66 (0.58–0.76)	0.57[a]
Serum aldosterone (pg/ml)	53.6 (20.0–100.0)	58.1 (24.4–120.0)	0.58[c]
Serum BNP (pg/ml)	83.1 (27.6–233.0)	68.6 (29.0–214.1)	0.62[c]
LV ejection fraction (%)	64.9±10.77	65.5±9.89	0.70[a]
LVMI ($g/m^{2.7}$)	51.6±21.08	52.3±18.10	0.82[a]
Women	45.4±19.79	51.2±14.28	0.30[a]
Men	53.9±21.26	52.8±19.41	0.79[a]
Medications			
ARBs, *n* (%)	68 (87.2)	73 (91.2)	0.15[b]
ACEIs, *n* (%)	10 (12.8)	7 (8.8)	0.60[b]
Calcium-channel blocker, *n* (%)	54 (74.0)	57 (71.3)	0.72[b]
α-Blocker, *n* (%)	13 (17.8)	18 (22.5)	0.55[b]
β-Blocker, *n* (%)	17 (23.3)	21 (26.3)	0.71[b]
Diuretics (furosemide), *n* (%)	53 (72.6)	39 (50.6)	0.01
Statin, *n* (%)	24 (32.9)	21 (26.3)	0.38
P binder, *n* (%)	44 (60.3)	49 (61.3)	1
EPO dose (U/wk)	24,000 (12,000–24,000)	24,000 (12,000–24,000)	0.79[c]

Values are expressed as number (percentage), mean±SD, and median (interquartile range). TC, total cholesterol; LDL-C, LDL cholesterol; β_2-MG, β_2-macroglobulin; BNP, brain natriuretic peptide; EPO, erythropoietin.
[a]Welch *t* test.
[b]Fisher exact test.
[c]Mann–Whitney *U* test.

during the follow-up period in the group given spironolactone but increased significantly in the control group at 12 (P=0.02 versus baseline; P=0.01 versus spironolactone treatment group), 18 (P<0.001 versus baseline; P<0.001 versus spironolactone treatment group), and 24 (P<0.001 versus baseline; P<0.001 versus spironolactone treatment group) months (Figure 2B). The rate of change in LVMI for men with LVMI>50 $g/m^{2.7}$ (LVH) at baseline did not significantly change during the observation period in the control group, but decreased significantly after 6 months when compared with baseline, and was further suppressed at 18 (P=0.001 versus baseline; P=0.003 versus control) and 24 (P<0.001 versus baseline; P=0.02 versus control) months by spironolactone (Figure 2C). In contrast, in women, rate of change in LVMI did not differ significantly during the

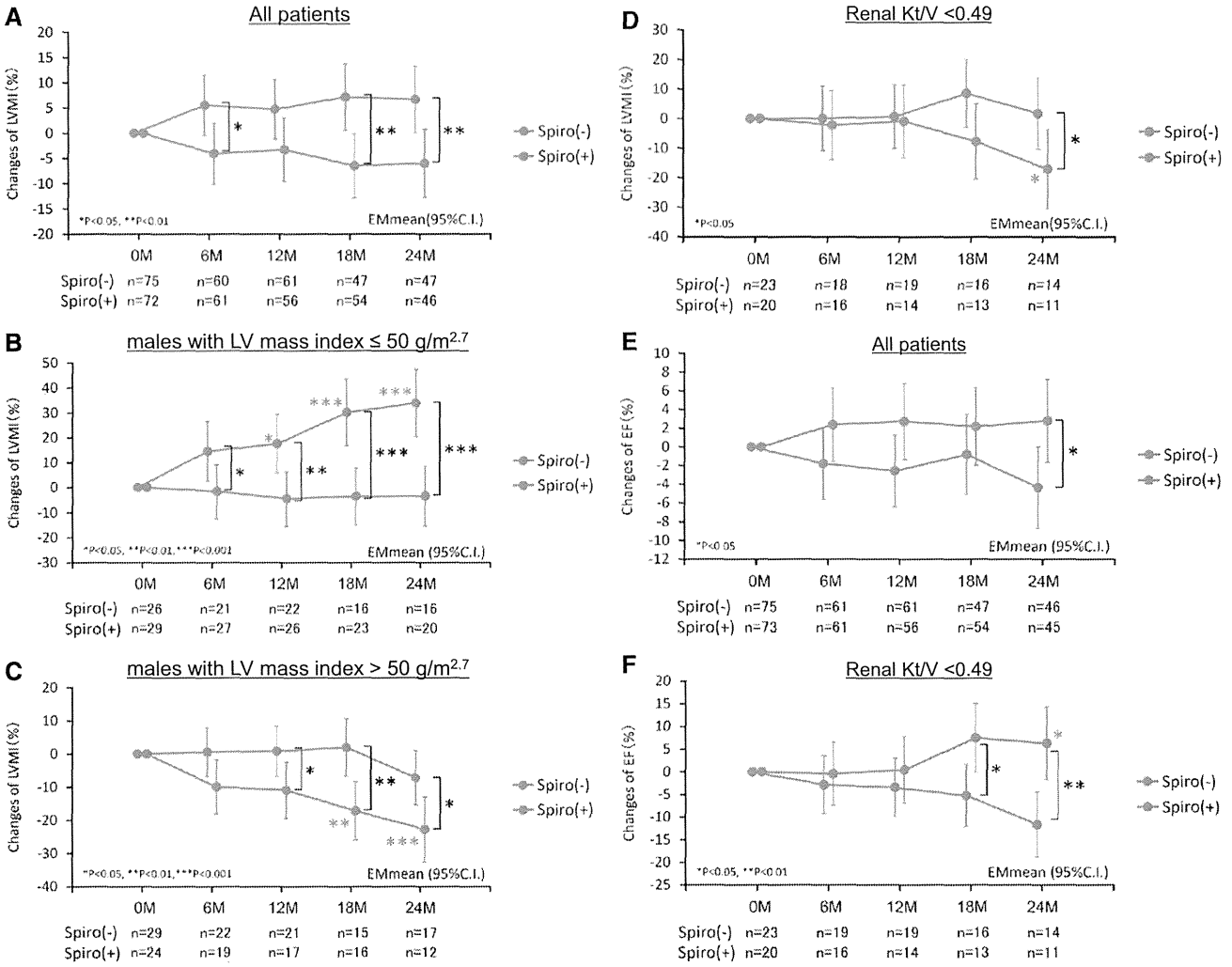

Figure 2. Spironolactone prevented cardiac hypertrophy and decreases in LVEF in patients on PD. Effects of spironolactone are clear in men and in patients with reduced residual renal function. Effects of spironolactone on LVMI and function in patients undergoing PD. LVMI in all patients (A), in men with LVMI≤50 g/m$^{2.7}$ (B) or >50 g/m$^{2.7}$ (C), and in PD patients with renal Kt/V<0.49 (D). Ejection fraction in all patients (E) and in patients with renal Kt/V<0.49 (F). 95% C.I., 95% confidence interval; Spiro (−), control group; Spiro (+), spironolactone treatment group.

observation period in patients with LVMI≤47 g/m$^{2.7}$ (normal range for women) or in those with LVMI>47 g/m$^{2.7}$ (LVH)[19,20] at baseline (Supplemental Figure 2).

Residual renal function was reported to affect LVMI in patients undergoing PD.[20–22] Patients were divided into tertiles according to baseline renal Kt/V: tertile 1, <0.49; tertile 2, ≥0.49 and <0.94; and tertile 3, ≥0.94. The effects of spironolactone were obvious in patients with renal Kt/V<0.49 (tertile 1) (Figure 2D), but were not significant in tertiles 2 and 3.

Changes in LVEF

Rate of change in LVEF was significantly improved at 24 weeks compared with the nontreatment control group (P=0.02) (Figure 2E). On analysis of changes of LVEF in tertiles of renal Kt/V, spironolactone improved LVEF at 18 (P=0.02 versus control) and 24 (P=0.002 versus control) months in the patients with Kt/V<0.49 (Figure 2F), but in tertiles 2 and 3, these effects were not detected.

Secondary Outcomes

Both urine volume and renal Kt/V decreased with time, but rate of change did not significantly differ between the treatment and control groups (Figure 3, A and B). Significant effects of spironolactone were not detected in the rate of change in D/P Cr ratio between the groups (Figure 3C).

Adverse Effects

A total of two patients in the spironolactone group (2.6%) and five patients in the control group (6.3%) died (Figure 1). Kaplan–Meier estimates of mortality and technical survival rate at 24 months were not significantly different (Supplemental Figure 3, A and B; P=0.34 and 0.34, respectively). Kaplan–Meier

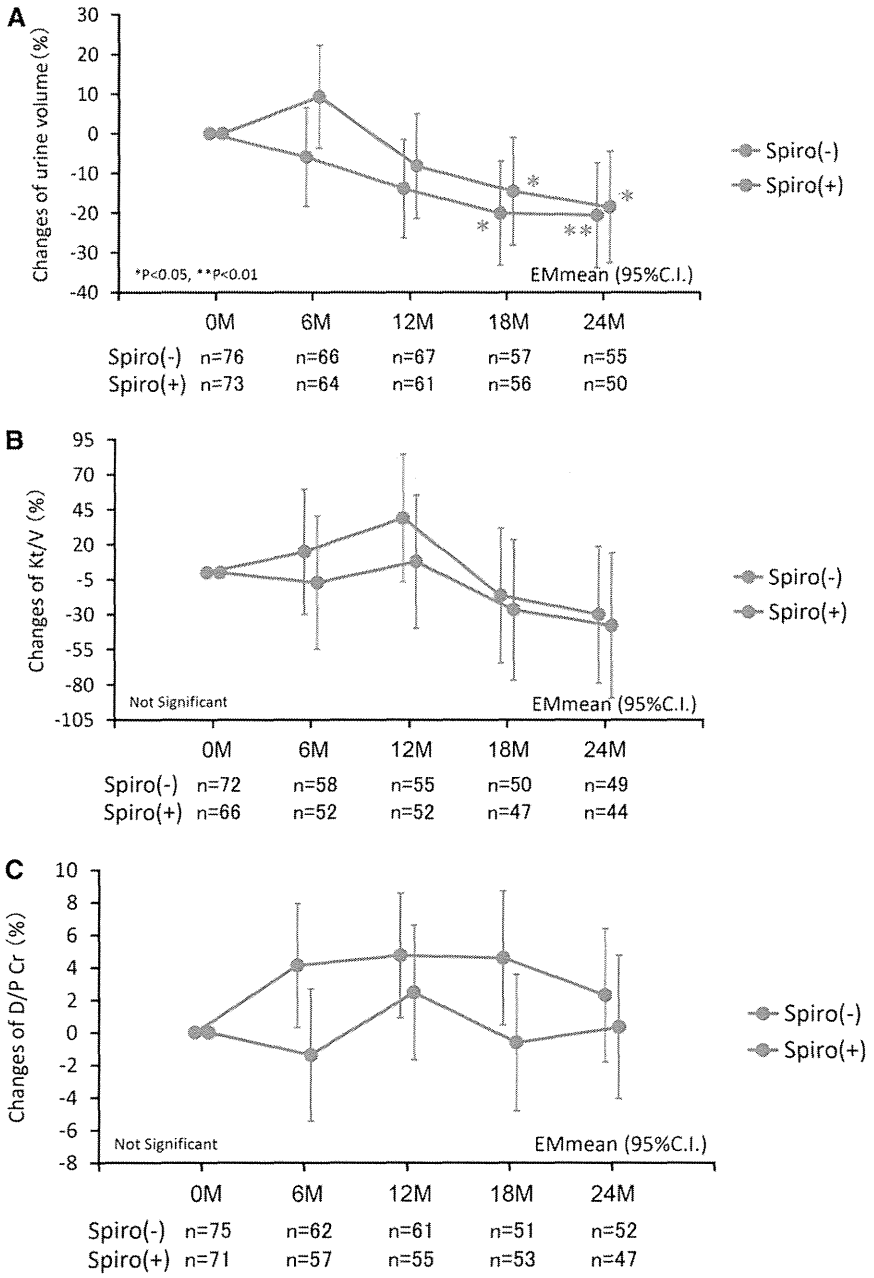

Figure 3. Both urine volume and renal Kt/V decreased with time, but rate of change did not significantly differ between the treatment and control groups. Effects of spironolactone on residual renal function and peritoneal membrane function. (A) Changes in urine volume. Rate of change in urine volume was significantly higher in both groups after 18 months compared with baseline values but did not significantly differ between groups during the observation period. (B) Changes in residual renal function Kt/V. Changes in renal Kt/V were not significantly different compared with baseline values and did not significantly differ between groups during the observation period. (C) Peritoneal membrane function assessed by D/P Cr. Rate of change in D/P Cr did not differ between groups. 95% C.I., 95% confidence interval; Spiro (−), control group; Spiro (+), spironolactone treatment group.

values for cardiovascular death and technical survival from cardiovascular causes did not significantly differ between the groups during the study period (Supplemental Figure 3, C and D; P=0.15 and 0.71, respectively). There were no significant differences between the two groups in occurrence of cerebral bleeding (P>0.99), cerebral infarction (P>0.99), myocardial infarction (P>0.76), and hypotension (P=0.76) (Table 2).

Serum potassium levels were significantly higher in patients receiving spironolactone at 6 and 12 months (Supplemental Figure 4). Serious hyperkalemia (potassium≥6.0 mEq/L) occurred in two (2.6%) patients (maximum level, 6.0 mEq/L) who received spironolactone and in one (1.3%) (maximum level, 6.2 mEq/L) in the control group during the observation period (P=0.62) (Table 2). Gynecomastia developed in 11 (14.1%) and 2 (2.5%) patients in the spironolactone and control groups, respectively (P=0.01) (Table 2). Spironolactone was switched to eplerenone in two patients, and the doses of spironolactone were reduced from 25 to 12.5 mg/d in two other patients. The remaining seven patients continued with spironolactone. There were no other clinically significant differences between the two groups with respect to laboratory variables, reported adverse events, or adverse events leading to permanent withdrawal of the study medicine.

DISCUSSION

Left ventricular hypertrophy is present in almost 75% of patients starting dialysis[23] and is strongly associated with poor outcomes in patients with CKD and ESRD.[1] Recently, much attention has focused on the mediators and signaling pathways for LVH. Activation of the renin-angiotensin system (RAS), oxidative stress, elevated asymmetric dimethyl arginine, low-grade inflammation with increased circulating cytokines, and dyslipidemia are reported to be involved in the pathogenesis of cardiovascular diseases in patients with CKD.[24] Persistent hyperaldosteronemia and/or activation of MR can promote cardiac fibrosis, possibly through generation of signals promoting profibrotic TGF-β production.[1,25,26] In animal models, spironolactone ameliorates cardiac fibrosis in nephrectomized uremic rats.[27,28]

Table 2. Adverse events

Adverse Event	Patients, *n* (%)		*P* Value
	Spironolactone Group (*n*=78)	Control Group (*n*=80)	
Cerebral bleeding	1 (1.3)	2 (2.5)	>0.99
Cerebral infarction	4 (5.1)	4 (5.0)	>0.99
Acute myocardial infarction	2 (2.6)	8 (10.0)	0.10
Hypotension (BP<100 mmHg)	6 (7.7)	5 (6.3)	0.76
Hyperkalemia (K>6.0 mEq/L)	2 (2.6)	1 (1.3)	0.62
Hypokalemia (P<3.0 mEq/L)	12 (15.4)	20 (25.0)	0.17
Gynecomastia	11 (14.1)	2 (2.5)	0.01
Peritonitis	16 (20.5)	17 (21.3)	>0.99

We evaluated the effects of adding spironolactone to ACEI or ARB in patients undergoing PD, and we found that LVMI was significantly suppressed in the spironolactone group (Figure 2). LVMI in patients receiving PD was reported to be linked to a loss of residual renal function[2,20] and to a high peritoneal membrane permeability.[29] Thus, the absence of significant effects from spironolactone on renal Kt/V and D/P Cr in this study (Figure 3) supports the notion that these results were mainly due to the effects of spironolactone. In subgroup analysis, the effects of spironolactone were more significant in men (Figure 2). On the other hand, we observed no effect from spironolactone in women (Supplemental Figure 2). In previous studies, in which about 30% of enrolled patients were women, spironolactone was effective in both male and female patients with severe heart dysfunction who were not undergoing dialysis.[10] Because of the low number of women in our study, a comparison of the effects of spironolactone between men and women in a larger cohort will be necessary to confirm our data. We did not observe significant interactions between the effect of spironolactone and residual renal functions on rate of changes in LVMI (*P* for interaction=0.43) or LVEF (*P* for interaction=0.18); however, we analyzed the relationship with residual renal function on the basis of its clinical importance for PD patients.[20–22] Interestingly, the effects of LVMI and LVEF tended to be predominant in PD patients with decreased levels of residual renal function (renal Kt/V<0.49) (Figure 2, D and F). These important points need to be confirmed in future studies.

As for secondary outcomes, we were unable to identify the beneficial effects of spironolactone on residual renal function and peritoneal membrane functions. In most clinical studies on MR blockade in patients with CKD, the primary endpoint has been reduction of proteinuria and/or albuminuria.[30–32] The effects of MR blockade in prevention of deterioration of renal function remain unclear.[30,31] In our studies, we were unable to identify the effects on prevention of decline in urine volume and residual renal function in patients undergoing PD. MR protein and mRNA were expressed in rat peritoneum, mesothelial cells, and fibroblasts.[33,34] Spironolactone and ARB effectively ameliorated the peritoneal thickening and D/P Cr in the rat scraped peritoneal fibrosis model.[33] In addition, ARB was reported to suppress the deterioration of peritoneal membrane function in PD patients.[35] In this study, RAS was suppressed at baseline in patients by administration of ACEI or ARB for >3 months. Therefore, in these cases, it may be difficult to detect the effects of spironolactone on peritoneal membrane function.

Overall, spironolactone therapy was well tolerated. The use of MR antagonists has not been recommended in patients with CKD because of concerns about hyperkalemia, which often occurs when multiple renin-angiotensin-aldosterone blockers are used[30,36,37]; however, the risk of severe hyperkalemia was not significantly elevated among PD patients assigned to spironolactone (Table 2). Removal of potassium is greater in PD than in hemodialysis. Between 10% and 36% of patients receiving PD are reported to have hypokalemia, and adding potassium tablets is necessary for 10%–20% of PD patients.[38–40] In our cohort, hypokalemia (potassium<3.0 mEq/L) was detected in 15.4% of the spironolactone treatment group and in 25% of the control group. On the basis of our findings, MR antagonists do not present serious problems with regard to serum potassium levels in patients undergoing PD. Antiandrogenic adverse effects appeared with high doses of spironolactone,[30,41] and a significant incidence of gynecomastia was noted in the spironolactone group (14.1%; *P*=0.01); however, in these cases the spironolactone dose was reduced from 25 mg/d to 12.5 mg/d or the patients were switched to eplerenone. Use of the selective MR blocker eplerenone appears to minimize the risk of gynecomastia.

This study had several limitations. First, this study was open label. Second, we estimated sample size according to the effects of spironolactone on LVMI; thus, analyzing sex effects and other endpoints in this sample may be inappropriate. Third, all patients in the study were Japanese. Japanese patients are reported to have a better prognosis than comparable patients in the United States and Europe, and subclinical atherosclerosis, coronary disease mortality, and risk of coronary calcification are lower in Japanese persons.[42] Fourth, an ACEI or ARB was administered for >3 months; therefore, the RAS was already suppressed at the start of this study in patients of both groups. In addition, several types of ACEI and ARB were used, and we did not evaluate their doses. Furthermore, the prescription rate for ARBs was much higher than that for ACEIs in this cohort; this is typical in Japan[43] but is very different from patterns in the United States and European countries. The high incidence of cough among Asian individuals[44] may be related to the lower use of ACEI in this cohort and in Japanese hypertensive patients. Fifth, most of the patients in this study were classified as New York Heart Association functional class (NYHA-FC) I, which may have contributed to the lower mortality rate. Finally, the effects of spironolactone in women were not studied in detail because the number of female patients enrolled was

limited (<30%) in this cohort. Furthermore, there was an imbalance between the two groups with regard to baseline LVMI in women (Table 1) because randomization was performed before echocardiography.

In summary, the MR antagonist spironolactone may help to prevent cardiac hypertrophy and dysfunction in patients undergoing PD without significant adverse effects. Future studies are necessary in larger cohorts to confirm the effects of spironolactone with regard to relative effectiveness in women and men and the relationship between residual renal function and secondary endpoints.

CONCISE METHODS

Study Design

The Nagoya Spiro Study is a multicenter, open-label, prospective, randomized controlled trial conducted in 12 hospitals. Patients were randomly assigned and followed for 2 years. This study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The institutional review boards or ethics committees of all participating institutions approved the protocols. Written informed consent was obtained from all patients before enrollment. The trial was monitored by an independent data and safety monitoring committee. Safety was evaluated by adverse events reports and clinical laboratory values, including serum potassium levels. The Nagoya Spiro Study was registered at The University Hospital Medical Information Network Clinical Trials Registry as UMIN000492.

Participants

Patients with ESRD undergoing PD were recruited from 12 hospitals in the Tokai area of Japan, including Nagoya University Hospital (Nagoya, Japan), Handa Municipal Hospital (Handa, Japan), Daiyukai Daiichi Hospital (Ichinomiya, Japan), Yokkaichi Municipal Hospital (Yokkaichi, Japan), Anjo Kosei Hospital (Anjo, Japan), Aihoku-Konankosei Hospital (Konan, Japan), Nagoya Kyoritsu Hospital (Nagoya, Japan), Gifu Prefectural Hospital (Gifu, Japan), Tosei General Hospital (Seto, Japan), Ogaki Kita Clinic (Ogaki, Japan), and Kasugai Municipal Hospital (Kasugai, Japan). Inclusion criteria were as follows: age 18–80 years, treatment with PD among patients with NYHA-FC I and II for <5 years, concurrent administration of ARB or ACEI treatment for >3 months, and treatment with neutral-pH dialysate and/or icodextrin solution. Patients were excluded if any of the following criteria were present: severe anemia (hemoglobin<9.0 g/dl), NYHA-FC III and IV heart failure, treatment with acid-pH fluid dialysate, previous treatment with hemodialysis or transplantation within the preceding 3 years, and pregnancy or suspected pregnancy.

Treatment Procedures

The 25-mg once-daily dose of spironolactone was selected on the basis of previous results of clinical trials.[10,32] If hyperkalemia or gynecomastia developed, the dose could be decreased to 12.5 mg/d because spironolactone at a dose 12.5–25 mg/d is reported to be pharmacologically effective in blocking the aldosterone receptors and atrial natriuretic peptide concentrations.[10,45] In addition, antihypertensive agents, excluding other ACEIs, ARBs, and MR blockers, were allowed to control systolic BP (<130 mmHg). Doses of ACEIs and ARBs were not allowed to change unless adverse events occurred. Following the approval of eplerenone for use in clinical settings in Japan in July 2007, the Nagoya University Institutional Review Board approved the change to eplerenone, 50 mg/d, from spironolactone after demonstration of the safety of spironolactone on serum potassium levels in this study. Follow-up evaluation and laboratory measurements, including serum potassium, were conducted every 4 weeks for up to 2 years.

Outcome Measures

The primary outcome measures were LVMI and LVEF. Secondary outcome measures comprised residual renal function and peritoneal membrane function. We evaluated LVMI and LVEF by echocardiography, renal Kt/V, urine volume, and D/P Cr by peritoneal equilibration test every 6 months for up to 24 months.

Echocardiography

Standard two-dimensional (2D) and Doppler echocardiography was performed in each institute using a commercially available echocardiographic machine with a 2.0- to 3.5-MHz transducer by a single experienced echocardiography sonographer. All echocardiographic data were recorded in accordance with the guidelines of the American Society of Echocardiography (ASE).[46,47]

Interventricular septal thickness (IVS), posterior wall thickness (PWT), and LV internal dimension (LVID) were measured at end-diastole and end-systole according to the established standards of ASE. To obtain optimal medial–lateral beam orientation and accurate linear measurements, the protocols recommend recording and measuring IVS, PWT, and LVID from the parasternal long-axis acoustic window using 2D-targeted M-mode echocardiography. In addition, observation from the parasternal short-axis acoustic window is required. Measurements of LVID and wall thickness are recommended at the level of mitral valve leaflet tips. Scanned images of 2D long-axis and short-axis views of end-diastolic and end-systolic ventricle with M-mode scans are required to send to the evaluation center.

LV mass was calculated according to the ASE-recommended formula[47,48]:

$$\text{LV mass (g)} = 0.8\times\left\{1.04\left[(\text{IVS}+\text{LVID}+\text{PWT})^3-(\text{LVID})^3\right]\right\}+0.6$$

LV mass was indexed by height to minimize potential distortion by extracellular volume expansion, and LVMI was defined as LV mass/height$^{2.7}$.[19,20] LVH was defined as LVMI>50 g/m$^{2.7}$ in men and 47 g/m$^{2.7}$ in women.[19,20] LVEF was obtained using the modified biplane Simpson method from the apical two- and four-chamber views. Echocardiography sonographers and interpreters were blinded to treatment assignment and protocol. All echocardiographic data were sent to the evaluation center and were reviewed in a blinded manner by a cardiologist (T.M.) to confirm the suitability and validity of echocardiography. In these procedures, data from four patients were omitted because of poor evaluation.

Sample Size

According to previous studies on LVH regression by eplerenone in hypertensive patients without advanced renal dysfunction,[13] we estimated a −30% reduction in LVMI for 2 years by adding the spironolactone to an ACEI or ARB. It was estimated that a total of 105 patients was necessary to detect significant differences between the two groups with 90% power. The rate of PD dropout from all causes is 10%–20% per year in Japan. To compensate for dropout from this study, we planned to enroll and randomly assigned at least 145 patients.

Statistical Analyses

The trial population in this study is the full analysis set population. Comparison between two groups of continuous variables was performed using the Welch t test or the Mann–Whitney U test. The Fisher exact test was used for categorical variables. Death and technical survival were analyzed by the Kaplan–Meier method and log-rank test. We used estimated marginal means with the linear mixed-effect model with Bonferroni correction to assess group differences in percentage change in LVMI. For the linear mixed-effect model, we included comparison groups and times as fixed effects and patients as random effects. We separately analyzed the effects of spironolactone between men and women, and also between normal LV mass and LVH. Effects of spironolactone were assessed by tertile of residual renal function. Tests of between-subject effects with type III sum of squares were assessed for interactions between fixed effects. We adjusted all analyses with baseline use of diuretics (furosemide). Differences were considered to be statistically significant at $P<0.05$. All analyses were performed using SPSS Statistics 20 (IBM Corp., Armonk, NY).

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DISCLOSURES

None.

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