Accumulation of advanced glycation end products in the peritoneal vasculature of continuous ambulatory peritoneal dialysis patients with low ultra-filtration

Kazuho Honda¹, Kosaku Nitta¹, Shigeru Horita¹, Wako Yumura¹, Hiroshi Nihei¹, Ryoji Nagai², Kazuyoshi Ikeda¹ and Seikoh Horiuchi²

¹Department of Medicine, Kidney Center, Tokyo Women’s Medical University, Tokyo and ²Department of Biochemistry, Kumamoto University School of Medicine, Kumamoto, Japan

Abstract

Background. Ultra-filtration failure is a serious complication of long-term continuous ambulatory peritoneal dialysis (CAPD). This complication is related to histological changes of the peritoneum, i.e. severe interstitial fibrosis and microvascular sclerosis. Although their pathogenesis has not been elucidated yet, advanced glycation end products (AGEs) have been shown to accumulate in the peritoneal tissue of CAPD patients.

Methods. Peritoneal biopsy specimens from 14 CAPD patients with low ultra-filtration (n=9) and high ultra-filtration (n=5) capacity were immunohistochemically investigated using a monoclonal antibody against AGEs (6D12). The severity of peritoneal fibrosis, microvascular sclerosis and intensity of AGE accumulation were semi-quantitatively evaluated. Peritoneal ultra-filtration capacity was evaluated by calculating daily ultra-filtration volume per body weight (UFV/BW) and D/D₀ (glucose) of the peritoneal equilibration test.

Results. In all patients with low ultra-filtration, AGE accumulated in the peritoneal fibrous tissue and microvascular walls. Remarkably, AGE accumulated more intensely in hyalinized fibrosis of small venular media. Extent of AGE accumulation in peritoneal interstitium and vascular walls correlated with the progression of interstitial fibrosis (ρ=0.727, P=0.0088) and vascular sclerosis (ρ=0.915, P=0.001). UFV/BW was inversely correlated to interstitial fibrosis (ρ=-0.660, P=0.0174), microvascular sclerosis (ρ=-0.671, P=0.0155) and microvascular AGE accumulation (ρ=-0.678, P=0.0145).

Conclusions. In CAPD patients, AGE formation in the peritoneum correlates with the development of severe interstitial fibrosis and microvascular sclerosis, which is associated clinically with impaired peritoneal ultra-filtration.

Key words: advanced glycation end product; continuous ambulatory peritoneal dialysis; peritoneal fibrosis; ultra-filtration failure; vascular changes

Introduction

Advanced glycation end products (AGEs) are formed by a non-enzymatic reaction between reduced sugar and protein, known as the Maillard reaction [1,2]. AGEs accumulate on serum proteins and various tissue proteins in patients with diabetes mellitus (DM), suggesting that they play a role in the pathogenesis of diabetic complications [3–6]. Recently, several reports have disclosed AGE accumulation in sera and tissues of chronic renal failure (CRF) patients, irrespective of the presence or absence of DM [7–10]. In CRF patients, AGEs are formed due to high oxidative stress associated with the uraemic state [11]. The high concentration of glucose in the peritoneal dialysate of continuous ambulatory peritoneal dialysis (CAPD) patients has been shown to facilitate AGE formation in the peritoneal membrane [9,12].

We recently described the marked peritoneal vascular changes in CAPD patients with ultra-filtration failure, i.e. severe fibrosis and hyalinization of the media of small venules [13]. The present study was designed to evaluate the contribution of AGE in the development of peritoneal lesions associated with ultra-filtration failure. Semi-quantitative analysis disclosed that the severity of peritoneal changes were positively correlated with the grade of AGE accumulation. Furthermore, an inverse relationship between peritoneal ultra-filtration capacity represented by daily ultra-filtration volume per body weight (UFV/BW) and histological changes was also observed. In addition, the pathogenesis of AGE related peritoneal changes, especially microvascular sclerosis, is discussed.
Patients and methods

Patients

Fourteen patients who interrupted their CAPD treatment were examined. The reasons of CAPD interruption were ultra-filtration failure (four cases), peritonitis (four cases), cerebrovascular disease (three cases), spinal injury (one case), destructive spondyloarthropathy (one case) and electrolyte disturbance (one case). All patients provided informed consent to participate in this study which was approved by the regional scientific ethics committee. Four patients (patient no. 2, 4, 9 and 14) were identical to the patients in our previous study [13]. The clinical data of the patients are summarized in Table 1. The mean age of the patients was 50.9 years (range 23–66 years) and the mean duration of CAPD was 77.6 months (range 16–144 months). The primary renal disease of all patients was chronic glomerulonephritis.

Clinical evaluation of peritoneal ultra-filtration capacity

Among 14 patients, nine had ultra-filtration loss which we defined as a reduced ultra-filtration volume less than 800 ml/day, although high osmolar dialysate, 2.5% Dianiel (Baxter) or Perisate 400 (Japan Medical Supply), was used more than twice a day. These nine patients composed the low ultra-filtration (low UF) group and the other five patients were included into the high ultra-filtration (high UF) group. A peritoneal equilibration test (PET) [14] was performed in 12 out of the 14 patients. The ratio of dialysate glucose concentration, $D/D_0$ ($D_0$ at start, $D$ at 4 h) and the ratio of plasma and dialysate creatinine concentration, $P/D$ ($P$, plasma; $D$, dialysate) were calculated, which are indicative of ultra-filtration capacity and the permeability of low molecular weight solutes, respectively. Daily ultra-filtration UFV/BW and $D/D_0$ of PET were used as indicators of peritoneal ultra-filtration capacity. The relationship between UFV/BW and $D/D_0$ was also analysed. Furthermore, we compared the clinical data of low and high UF groups.

Histological and immunohistochemical methods

Parietal peritoneum specimens were obtained during surgery to remove the catheter. The peritoneal tissues were fixed with 10% phosphate-buffered formalin (pH 7.2), embedded in paraffin and cut into 4 μm sections and then stained with haematoxylin & eosin and Mallory–Azan for light microscopy. For the immunohistochemical study, paraffin sections on glass slides coated with gelatin were stained with a peroxidase-labelled streptavidin-biotin staining kit (DAKO Co., CA, USA). The primary antibodies were monoclonal anti-CML antibody (6D12) [15,16]. Replacement of the primary antibodies with irrelevant anti-mouse IgG antibodies served as control.

![Fig. 1. Grading of peritoneal interstitial fibrosis. Grade 0, no interstitial fibrosis (A); grade 1, mild interstitial fibrosis (B); grade 2, moderate interstitial fibrosis with thickening of peritoneum (C); and grade 3, severe interstitial fibrosis with marked thickening of peritoneum (D). (Mallory–Azan stain, × 15).](image-url)
Table 1. Clinical profiles and grading of histological changes and AGE accumulation in the peritoneum

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age</th>
<th>Sex</th>
<th>BW (kg)</th>
<th>CAPD duration (month)</th>
<th>Reason of CAPD interruption</th>
<th>Dialysate (kind of dialysate, volume × times/day)</th>
<th>Ultra-filtration volume (ml/day)</th>
<th>D/D₀(PET) (glucose)</th>
<th>D/P(PET) (creatinine)</th>
<th>Interstitial fibrosis</th>
<th>Interstitial AGE</th>
<th>Vascular sclerosis</th>
<th>Vascular AGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low UF group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>58 m</td>
<td></td>
<td>57</td>
<td>144</td>
<td>peritonitis</td>
<td>Dianiel 1.5% 2.0 l × 2, Dianiel 2.5% 2.0 l × 2</td>
<td>600</td>
<td>0.36 (HA)</td>
<td>0.74 (HA)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>58 m</td>
<td></td>
<td>67</td>
<td>108</td>
<td>apoplexy</td>
<td>Dianiel 2.5% 2.0 l × 4, Dianiel 2.5% 2.0 l × 4 × 2</td>
<td>700</td>
<td>0.24 (H)</td>
<td>0.89 (H)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>23 m</td>
<td></td>
<td>61</td>
<td>84</td>
<td>UFF</td>
<td>Perisate 400 2.0 l × 4, 200</td>
<td>200</td>
<td>0.19 (H)</td>
<td>0.86 (H)</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>40 m</td>
<td></td>
<td>62</td>
<td>75</td>
<td>UFF</td>
<td>Dianiel 2.5% 2.0 l × 4, 800</td>
<td>800</td>
<td>0.34 (HA)</td>
<td>0.68 (HA)</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>54 m</td>
<td></td>
<td>68</td>
<td>74</td>
<td>peritonitis</td>
<td>Perisate 360 2.0 l × 4, Perisate 400 2.0 l × 1</td>
<td>500</td>
<td>0.22 (H)</td>
<td>0.86 (H)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>66 m</td>
<td></td>
<td>62</td>
<td>72</td>
<td>UFF</td>
<td>Perisate 360 1.5 l × 1, Perisate 400 1.5 l × 4</td>
<td>600</td>
<td>0.17 (H)</td>
<td>0.91 (H)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>39 m</td>
<td></td>
<td>46</td>
<td>68</td>
<td>UFF</td>
<td>Dianiel 1.5% 1.5 l × 1, Dianiel 2.5% 1.5 l × 4</td>
<td>600</td>
<td>0.33 (HA)</td>
<td>0.68 (HA)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>58 f</td>
<td></td>
<td>62</td>
<td>66</td>
<td>DSA</td>
<td>Dianiel 1.5% 1.5 l × 2, Dianiel 2.5% 1.5 l × 4</td>
<td>800</td>
<td>0.37 (HA)</td>
<td>0.77 (HA)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>44 f</td>
<td></td>
<td>40</td>
<td>47</td>
<td>UFF</td>
<td>Perisate 360 1.5 l × 2, Perisate 400 1.5 l × 3</td>
<td>100</td>
<td>0.21 (H)</td>
<td>0.85 (H)</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>High UF group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>58 f</td>
<td></td>
<td>47</td>
<td>126</td>
<td>apoplexy</td>
<td>Dianiel 1.5% 1.5 l × 3, Dianiel 2.5% 1.5 l × 2</td>
<td>1000</td>
<td>0.38 (HA)</td>
<td>0.78 (HA)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>50 m</td>
<td></td>
<td>62</td>
<td>94</td>
<td>apoplexy</td>
<td>Dianiel 1.5% 2.0 l × 2, Dianiel 2.5% 2.0 l × 2</td>
<td>1300</td>
<td>not done</td>
<td>not done</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>40 f</td>
<td></td>
<td>64</td>
<td>84</td>
<td>peritonitis</td>
<td>Perisate 360 2.0 l × 2, Perisate 400 2.0 l × 2</td>
<td>1600</td>
<td>0.48 (LA)</td>
<td>0.6 (LA)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>66 m</td>
<td></td>
<td>63</td>
<td>29</td>
<td>electrolyte disorder</td>
<td>Dianiel 1.5% 2.0 l × 4</td>
<td>1600</td>
<td>0.46 (LA)</td>
<td>0.71 (HA)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>58 m</td>
<td></td>
<td>64</td>
<td>16</td>
<td>spinal injury</td>
<td>Perisate 360 2.0 l × 4</td>
<td>1400</td>
<td>not done</td>
<td>not done</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

BW, body weight; UFF, ultra-filtration failure; CGN, chronic glomerulonephritis; DM, diabetes mellitus; DSA, destructive spondyloarthropathy; PET, peritoneal equilibration test; H, high; HA, high average; L, low; LA, low average.
Histological and immunohistochemical grading

A semi-quantitative system was used to grade the extent of interstitial and vascular fibrosis involving the peritoneum. Interstitial fibrosis was divided into four grades: grade 0, no interstitial fibrosis; grade 1, mild interstitial fibrosis; grade 2, moderate interstitial fibrosis with thickening of the peritoneum; and grade 3, severe interstitial fibrosis with marked thickening of the peritoneum (Figure 1A–D). Microvascular change was also divided into four grades: grade 0, no vascular abnormality; grade 1, mild perivascular fibrosis without stenosis of the lumen; grade 2, moderate perivascular fibrosis and thickening of the vascular wall with mild to moderate stenosis of the lumen; and grade 3, severe perivascular fibrosis with marked stenosis or occlusion of the lumen (Figure 2A–D). AGE accumulation in the interstitium and vasculature was evaluated by the same semi-quantitative grading system: grade 0, no AGE accumulation; grade 1, mild; grade 2, moderate; and grade 3, severe AGE accumulation in interstitium (Figure 3A–D) and vasculature (Figure 4A–D). We assessed correlations between histological changes and AGE accumulation and between the pathological changes and the peritoneal ultra-filtration capacity indicated by UFV/BW and D/D₀ (glucose) of PET.

Statistical analysis

The Mann–Whitney U test was used to compare the differences in the clinical data and the grade of histological alterations scores between low UF and high UF groups. The Spearman rank correlation test was used to analyse the correlation between the grading results of histological and immunohistochemical findings and between the peritoneal alterations and ultra-filtration capacity. Significance was taken as $P < 0.05$ in all analyses.

Results

Clinical evaluation of peritoneal ultra-filtration capacity

Clinical profiles of the patients are shown in Table 1 and the differences in peritoneal ultra-filtration capacity between high and low UF groups are shown in Table 2. The daily ultra-filtration volume was $544 \pm 246$ ml/day in the low UF group and $1380 \pm 249$ ml/day in the high UF group ($P < 0.0001$). UFV/BW was $9.18 \pm 4.02$ ml/kg and $22.9 \pm 2.13$ ml/kg in the low and high UF groups, respectively ($P < 0.0001$). D/D₀ (glucose) of PET was $0.27 \pm 0.08$ in low UF group and $0.44 \pm 0.05$ in high UF group ($P = 0.0065$). Whereas, D/P (creatinine) of PET was not different between the two groups. UFV/BW was clearly correlated to D/D₀ (glucose) ($r = 0.888$, $P < 0.0001$, figure is not shown).
Fig. 3. Grading of AGE accumulation in peritoneal interstitium. Grade 0, no AGE accumulation (A); grade 1, mild (B); grade 2, moderate (C); grade 3, severe AGE accumulation (D). (6D12 staining, ×15).

Fig. 4. Grading of AGE accumulation in peritoneal vascular wall. Grade 0, no AGE accumulation (A); grade 1, mild (B); grade 2, moderate (C); grade 3, severe AGE accumulation (D). (6D12 staining, ×95).
Table 2. Comparison of ultra-filtration capacity between low and high ultra-filtration groups

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Low UF group</th>
<th>High UF group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>gender (m/f)</td>
<td>2/7</td>
<td>3/2</td>
<td>n.s.</td>
</tr>
<tr>
<td>age (years)</td>
<td>48.9 ± 13.4</td>
<td>54.4 ± 9.8</td>
<td>n.s.</td>
</tr>
<tr>
<td>CAPD duration (months)</td>
<td>82.0 ± 28.3</td>
<td>69.8 ± 46.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>Daily UFV (ml/day)</td>
<td>544 ± 246</td>
<td>1380 ± 249</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>UF/VBW (ml/kg)</td>
<td>9.18 ± 4.02</td>
<td>22.9 ± 2.13</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>D/D0 (glucose)</td>
<td>0.27 ± 0.08</td>
<td>0.44 ± 0.05</td>
<td>0.0065</td>
</tr>
<tr>
<td>D/P (creatinine)</td>
<td>0.80 ± 0.09</td>
<td>0.70 ± 0.09</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

UF, ultra-filtration; UFV, ultra-filtration volume; BW, body weight; D/D0, ratio of dialysate glucose concentration at start and 4 h; D/P, ratio of dialysate and plasma creatinine concentration at 4 h; n.s., not significant.

Grading of histological changes and AGE accumulation in the peritoneum

The grading of interstitial fibrosis, microvascular sclerosis, interstitial AGE accumulation and microvascular AGE accumulation in the peritoneum are listed in Table 1. The advanced grade of interstitial fibrosis (grade 1–3) was present more often in the patients with low UF than in those with high UF who showed mild interstitial fibrosis (grade 0–2). Similarly, the advanced grade of microvascular sclerosis (grade 1–3) was more frequent in the low UF patients than those with high UF who had no abnormality or mild degree of microvascular sclerosis (grade 0–2). Immuno-histochemical examination using monoclonal anti-AGE antibody (6D12) revealed AGE accumulation in the interstitium (Figure 3) and in the microvascular wall (Figure 4). AGE staining in the interstitial fibrosis was observed in all of the CAPD patients, although the extent of staining was variable. The patients with low UF showed intense AGE accumulation in the lesions of severe interstitial fibrosis (Figure 3C and D), whereas those with high UF showed AGE accumulation only in limited areas (Figure 3B). AGE also accumulated in the peritoneal vascular walls which were affected by sclerosis (Figure 4B–D), whereas no AGE accumulated in the microvessels without sclerosis (grade 0) (Figure 4A). The AGE accumulation in the vascular wall became more intense as the vascular sclerosis became more severe (Figure 4B–D). The comparison of the grading score of histological alterations and AGE accumulation is shown in Table 3. The grades of peritoneal interstitial fibrosis, microvascular AGE accumulation were significantly more advanced in the low UF group than in the high UF group, whereas the grade of interstitial AGE accumulation was not significantly different.

Correlation between the histological changes and AGE accumulation

The extent of interstitial fibrosis correlated positively with that of interstitial AGE accumulation (ρ = 0.727, P = 0.0088) (Figure 5A), and the extent of vascular sclerosis correlated with that of vascular AGE accumulation (ρ = 0.915, P = 0.001) (Figure 5B).
Fig. 6. Correlation between peritoneal alterations and daily ultra-filtration UFV/BW. (A) interstitial fibrosis and UFV/BW ($\rho = -0.660, P = 0.0174$), (B) interstitial AGE accumulation and UFV/BW ($\rho =$ n.s.), (C) microvascular sclerosis and UFV/BW ($\rho = -0.671, P = 0.0155$), (D) microvascular AGE accumulation and UFV/BW ($\rho = -0.678, P = 0.0145$).

cular sclerosis was inversely correlated to UFV/BW ($\rho = -0.671, P = 0.0155$) (Figure 6C), and the grade of microvascular AGE accumulation was inversely correlated to UFI ($\rho = -0.678, P = 0.0145$) (Figure 6D). D/D$_0$ (glucose) of PET did not significantly correlate observed in CAPD patients. In the present study, AGE was broadly accumulated in the peritoneal interstitium with the grade of histological changes and AGE accumulation (figures not shown).

Discussion

The morphological changes of the peritoneum in CAPD patients are mesothelial denudation, interstitial fibrosis resulting in thickening of the peritoneum and vascular alterations [17]. The most characteristic change is replication of the basement membrane of the peritoneal capillaries [18,19], similar to diabetic microangiopathy. We reported peritoneal microvascular changes in patients on long-term CAPD with ultrafiltration failure [13]. Light microscopically, severe fibrosis and hyalinization of the vascular media were observed, especially in post-capillary vessels to small venules. Electron microscopy revealed increased collagen fibres in the media of the vasculature with degenerative smooth muscle cells showing scant cytoplasm and pyknotic nuclei. These results suggest that certain factors could exert a toxic effect on vascular smooth muscle cell of the peritoneal vasculature.

Because the morphological changes of the peritoneal vasculature are similar to those associated with diabetic microangiopathy, we examined AGE accumulation in the peritoneal tissue to elucidate the contribution of AGE in the characteristic peritoneal alterations observed in CAPD patients. In the present study, AGE was broadly accumulated in the peritoneal interstitium in all patients and the progression of interstitial fibrosis correlated positively with the extent of AGE accumulation (Figures 3 and 5A). On the other hand, AGE accumulation in the peritoneal vasculature was demonstrated only in the sclerotic vessels and the grade of vascular sclerosis correlated positively with the extent of vascular AGE accumulation (Figures 4 and 5B). These results suggest that AGE accumulation contributed to the interstitial fibrosis and the unusual vascular lesions observed in the CAPD patients.

In the peritoneum of CAPD patients, glycation of the peritoneal proteins might be facilitated by a high glucose concentration of the peritoneal dialysate [20]. In addition, high oxidative stress associated with the uraemic state also has a potential to increase the formation of AGES in the peritoneum [11]. AGE accumulation in systemic vessels in uraemic patients was demonstrated in the radial artery and renal vasculature [9]. However, the microvascular lesions observed in the peritoneum in CAPD patients are unique and were never found in any organs other than peritoneum. This finding suggests that the peritoneal microvascular lesions might be related to the specific
condition of CAPD, an exposure to a high concentration of glucose used in peritoneal dialysate.

It has been suggested that AGE induces macrophage chemotaxis and the release of TNF alpha, interleukin-1 and platelet-derived growth factor (PDGF) [21, 22]. These mediators activate vascular smooth cells to synthesize extracellular matrix components, resulting in severe fibrosis of the vascular wall. Alternately, AGE may induce structural and functional alterations of basement membrane components such as type IV collagen [23] and laminin [24], possibly contributing to the increased permeability of the affected microvessels. Furthermore, AGE modification of basement membrane components may inactivate endothelial cell-derived nitric oxide, a potent endothelium-derived vasodilator and anti-proliferative factor, leading to accelerated vascular and renal dysfunction in diabetic patients [25].

The clinical evaluation of peritoneal ultra-filtration capacity is technically difficult. Twardowski proposed the PET to evaluate the peritoneal membrane function and suggested that the ratio of dialysate glucose concentration before and after PET (D/D0) was a useful marker of peritoneal ultra-filtration capacity [14]. In this study, we employed D/D0 and UFV/BW as quantitative markers of peritoneal ultra-filtration capacity, and the inverse correlation between UFV/BW and peritoneal histological changes was demonstrated. This finding suggests that peritoneal fibrosis and microvascular sclerosis are associated with ultra-filtration failure. The reason that no significant correlation between D/D0 and the histological changes was observed is due to the inadequacy of the PET method, in which the volume and the time of peritoneal lavage are limited, irrespective of patient body mass differences.

Nakayama et al. demonstrated that AGE accumulation in the peritoneal vascular walls increased with the duration of CAPD treatment, suggesting that AGE in the vascular wall plays a role in the increased permeability of the peritoneal membrane [12]. This notion is consistent with the present finding that AGE accumulation in the peritoneal vasculature is closely related to the clinical signs of ultra-filtration failure. It is not fully understood why an increased interstitial fibrosis and thickening of microvascular walls are accompanied by an increased permeability of small solutes (e.g. glucose). Further investigation is required concerning the relationship between microvascular morphology and its function.

In conclusion, we demonstrated a significant AGE accumulation in the peritoneum of CAPD patients which was diffuse in the interstitium and more intense in the microvascular lesions. The extent of AGE accumulation in the peritoneal interstitium and vasculature correlated positively with the extent of interstitial fibrosis and peritoneal vascular sclerosis. Furthermore, the grade of interstitial fibrosis and peritoneal vascular sclerosis were inversely correlated to peritoneal ultra-filtration capacity. These results suggest that an increase in AGE formation due to a high glucose dialysate induces the progression of peritoneal fibrosis and microvascular sclerosis resulting in an impaired ultra-filtration capacity of the peritoneal membrane.

Acknowledgements. The authors are grateful to Ms Mayuko Kawashima and Mr Hideki Nakayama for the excellent technical assistance. This work was supported by a research grant (097708569) from the Ministry of Education, Science and Culture of Japan.

References
18. Gotloib L, Bar-Sella P, Shostak A. Reduplicated basal laminasclerosis were inversely correlated to peritoneal ultra-filtration capacity. These results suggest that an Ultrastructural changes of reduplicated peritoneal basal mem-
19. Di Paolo N, Sacchi G. Peritoneal vascular changes in continuous

Received for publication: 26.3.98
Accepted in revised form: 15.2.99