Association between Asymmetric Dimethylarginine and Pentosidine in Dialysis Effluent of Peritoneal Dialysis Patients

——A possible intraperitoneal crosstalk between asymmetric dimethylarginine and advanced glycation end products in peritoneal dialysis patients——

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Objective: Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of endothelial nitric oxide synthase. Elevated serum ADMA concentration is associated with impaired vascular endothelial function. We examined the relationships of ADMA with pentosidine, a representative advanced glycation end product, cytokines and the markers of peritoneal inflammation, damage and repair in dialysate effluent of peritoneal dialysis patients.
Methods: Study design was cross-sectional. Twenty-eight peritoneal dialysis patients who were ≥ 18 years of age, had been on peritoneal dialysis for at least 3 months and had no history of renal transplantation were enrolled. Dialysis effluent and blood were sampled after 8 hours of peritoneal dialysis. Concentrations of ADMA, pentosidine, cytokines and the markers of peritoneal inflammation, damage and repair were determined in dialysis effluent. Blood samples were analyzed for routine laboratory parameters.
Results: The effluent ADMA level had a significant correlation with effluent pentosidine concentration ($R=0.511$, $P=0.005$), but not with interleukin-6, interleukin-8, transforming growth factor-α, hyaluronic acid, cancer antigen 125 or fibrinogen/fibrin degradation products.
Conclusion: In the light of available evidence, our results suggest that AGEs generated during dialysate dwelling alters ADMA metabolism in the peritoneal tissues, leading to ADMA accumulation in the peritoneal cavity.

Key words: advanced glycation end products, asymmetric dimethylarginine, endothelial nitric oxide synthase, peritoneal dialysis.

INTRODUCTION

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of endothelial nitric oxide (NO) synthase that inhibits the release of NO from arginine, thereby compromising endothelium-dependent vasodilatation and vascular tone homeostasis [1, 2]. High plasma ADMA levels have been found in patients with arteriosclerosis, coronary artery disease, peripheral vascular disease, hypertension, chronic cardiac failure, hypercholesterolemia, chronic renal failure or diabetes mellitus [1]. In patients with chronic kidney disease, serum ADMA levels have been shown to be an independent predictor of progression to dialysis and death [3, 4].

Advanced glycation end products (AGEs) are protein adducts formed with reactive carbonyl compounds *via* the carbonyl-amine chemistry [5]. Both reactive carbonyl compounds and AGEs are present in high concentrations in the circulation of patients with diabetes or chronic renal insufficiency [6]. Reactive carbonyl compounds are also present in heat-sterilized glucose-containing dialysis fluids in the form of glucose degradation products, such as methylglyoxal, glyoxal, and 3-deoxyglucosone [5]. During peritoneal dialysis, reactive carbonyl compounds diffuse into the peritoneal cavity and, together with those contained in dialysate, form AGEs [7, 8]. Resulting AGEs cause structural and functional peritoneal deterioration [7, 8].

High circulating AGE levels appear to coexist with elevated serum ADMA levels in some conditions including diabetes and chronic renal disease. In fact, a recent study reported an association between the serum levels of ADMA and AGE in nondiabetic chronic kidney disease patients [9]. Another study found that plasma ADMA levels were positively associated with serum AGE levels and inversely correlated with flow-mediated vasodilatation in end-stage renal failure patients due to diabetic nephropathy [10]. To date, some investigators have speculated on a crosstalk between ADMA and AGE levels [11].

Efficient peritoneal dialysis depends on the permeability of vessels in the peritoneum. ADMA, if present in the peritoneal cavity, may alter peritoneal vessel permeability and affect dialysis efficiency. However, there have been no reports on the intraperitoneal ADMA levels.

In this study, we measured ADMA levels in dialysis

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effluents of peritoneal dialysis patients and investigated its relationships with effluent AGEs, cytokines and the markers of peritoneal inflammation, damage and repair.

SUBJECTS AND METHODS

This study conforms to the provisions of the Declaration of Helsinki and was approved by the Institutional Review Board of Jinyukai-Kitasaito Hospital, Asahikawa,Japan. Informed consent was obtained from all participating patients before the study entry. This study is registered with the Clinical Trials Registry of the University Hospital Medical Information Network (registration ID, UMIN 000019741).

Subjects

This single-center cross-sectional study enrolled 28 patients who were undergoing (stable) peritoneal dialysis at Kita-Saito Hospital, Asahikawa, Japan from October 2007 to May 2009. Inclusion criteria were age ≥ 18 years and having been undergoing peritoneal dialysis for at least 3 months. Exclusion criteria were history of renal transplantation. The patients were using neutral pH dextrose-based dialysate (*Dianeal-N*; Baxter Ltd., Tokyo, Japan) except for four patients who were using icodextrin-based solution (*Extraneal*; Baxter Ltd.).

Sample collections and measurements

Dialysis effluent and blood were sampled after 8 hours of overnight dialysis using 1.36% glucose dialysate (*Dianeal N PD4 1.5*; Baxter Ltd.). The effluent samples were sent to a commercial laboratory (SRL, Tokyo, Japan) for the measurements of ADMA, interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), fibrinogen/fibrin degradation products as a marker of peritoneal inflammation, hyaluronic acid as a marker of peritoneal tissue repair [12], cancer antigen 125 (CA125) as a marker of peritoneal mesothelial cell mass [13], and pentosidine as a representative AGE. Blood samples were analyzed for routine laboratory parameters.

Peritoneal equilibration test was conducted at the time of effluent collection, and the dialysate-to-plasma ratio of creatinine (D/P creatinine) was reported.

Statistical analysis

Data were expressed as mean ± SD. Differences between two groups were tested using Student's *t*-test. Correlations were tested by Pearson's correlation coefficient analysis. All statistical analysis was done with SPSS software (Ver. 17.0) (SPSS Inc., Tokyo, Japan). *P* values < 0.05 were considered significant.

RESULTS

The mean age of the 28 patients was 67 years, and the mean duration of dialysis was 19 months (Table 1). The primary kidney disease was chronic glomerulonephritis in 9 patients, diabetic nephropathy in 12 patients, nephrosclerosis in 3 patients, polycystic kidney disease in 1 patient, and idiopathic membranous nephropathy in 3 patients. The mean effluent ADMA concentration was 0.415 nmol/mL, and the range was 0.210−0.840 nmol/mL. There was no significant difference in the effluent ADMA concentration between the patients with diabetes and those without (Student's *t*-test, $P = 0.658$) or between those using icodextrin-based dialysate and the others (Student's *t*-test, $P = 0.996$).

We examined the relationships of the effluent ADMA with effluent pentosidine, cytokines and markers of peritoneal inflammation, damage and repair (Table 2). The effluent ADMA level had a significant correlation with the effluent pentosidine level ($R = 0.511$, $P = 0.005$; Table 2 and Figure). No significant correlation was found between the effluent ADMA level and effluent IL-6, IL-8, TNF-α, hyaluronic acid, CA125 or fibrinogen/fibrin degradation products (Table 2).

DISCUSSION

In this study, we examined the relationships between ADMA and the markers of peritoneal inflammation, damage and repair in dialysis effluents from peritoneal dialysis patients. We found a significant positive correlation between the effluent ADMA and pentosidine levels.

Recent studies provide an insight into the molecular mechanism of ADMA accumulation and suggest a link between AGEs and ADMA. ADMA is generated *via* the action of protein methyltransferase (PRMT) and is degraded primarily by dimethylarginine dimethylaminohydrolase (DDAH) [14, 15]. In a rat model of diabetes, DDAH activity was decreased in association with an increase in plasma ADMA levels [16]. In a rat model of chronic renal insufficiency, both PRMT expression and ADMA levels were elevated while DDAH activity was decreased, and the increase in ADMA levels was reversed by adenovirus-mediated introduction of DDAH gene [17]. DDAH mRNA expression was decreased in cultured endothelial cells upon exposure to AGEs, and the decrease was prevented by anti-sense DNA against the receptor of AGEs [10]. Exposure of rat aortic rings to AGEs *in vitro* decreased DDAH activity and inhibited endothelium-dependent relaxation [18]. Administration of AGEs to rats caused decreases in aortic DDAH and NO synthase activities, and aortic rings isolated from those rats showed inhibited endothelium-dependent relaxation [19]. These changes were reversed by administration *in vivo* of aminoguanidine, an inhibitor of AGE formation [19]. In the light of these pieces of evidence, our findings suggest that AGEs generated during dialysate dwelling downregulates DDAH activity in the peritoneal tissues, leading to ADMA accumulation in the peritoneal cavity.

Alternatively, the intraperitoneal association of ADMA and pentosidine can be a consequence of parallel diffusion of ADMA and reactive carbonyl compounds, the precursors of AGEs, from uremic circulation to the peritoneal cavity. Studies have shown that reactive carbonyl compounds influx into the peritoneal cavity from uremic circulation during dialysate dwelling [7, 8]. Since the dialysate effluent was sampled after 8 hours of dwelling, we cannot deny the possibility that a fraction of effluent AGEs were formed with reactive carbonyl compounds originated from the circulation.

Table 1 Patients characteristics and blood and dialysis effluent parameters

	Mean	SD
Age (years)	67.59	14.40
Duration of peritoneal dialysis (months)	19.41	19.12
Weight (kg)	61.39	11.85
Height (cm)	158.69	9.53
Icodextrin-based dialysate use	4	
D/P creatinine	0.69	0.12
Cardiothoracic ratio (%)	52.78	6.11
Blood parameters		
White blood cells (/μL)	5900.45	2122.93
Red blood cells (10^4/μL)	299.52	72.72
Hemoglobin (g/dL)	9.74	1.28
Hematocrit (%)	29.40	4.16
Albumin (g/dL)	3.11	0.47
Blood urea nitrogen (mg/dL)	56.89	13.57
Creatinine (mg/dL)	9.05	3.15
Potassium (mEq/L)	4.53	0.60
Phosphorus (mEq/L)	5.26	1.28
β2-microglobulin (mg/L)	20.72	8.52
High-sensitivity C-reactive protein (ng/mL)	1908.57	1698.10
Effluent parameters		
Asymmetric dimethylarginine (nmol/mL)	0.42	0.16
Fibrinogen/fibrin degradation products (μg/mL)	4.81	2.55
Interleukin-6 (pg/mL)	77.62	124.44
Interleukin-8 (pg/mL)	230.14	478.29
Hyaluronic acid (ng/mL)	428.81	652.32
Cancer antigen 125 (U/mL)	26.83	23.57
Transforming growth factor-α (pg/mL)	8.89	13.60
Pentosidine (μg/mL)	0.01	0.01

Abbreviations: D/P creatinine, dialysate/plasma creatinine ratio.

Table 2 Relationships of effluent asymmetric dimethylarginine with dialysis effluent parameters by Pearson's correlation coefficient analysis

Factor	R2	P value
Effluent pentosidine	0.511	0.005
Interleukin-6	– 0.128	0.516
Interleukin-8	– 0.283	0.144
Transforming growth factor-α	0.373	0.051
Hyaluronic acid	0.020	0.920
Cancer antigen 125	0.018	0.926
Fibrinogen/fibrin degradation products	0.159	0.421

Nevertheless, our present findings are of significance on two accounts. First, we revealed the presence of ADMA in dialysis effluent of peritoneal dialysis patients. Given the established roles of ADMA in endothelial dysfunction [1, 2], our findings raise the possibility that intraperitoneal ADMA alters the function of peritoneal endothelium. Second, the intraperitoneal association and possible interactions of ADMA and pentosidine suggest that the measures against AGE formation can protect the peritoneum from both AGEs and ADMA. In this regard, Kakuta *et al.* [20] have shown that intraperitoneal administration of pyridoxamine, an inhibitor of AGE formation, can prevent progressive deterioration of the peritoneal membrane. More recently, Mori *et al.* [21] demonstrated that pyridoxamine would also be effective when orally administered.

LIMITATIONS

First, our study was a cross-sectional study which does not allow the elucidation of causal relationships between correlated parameters. Second, the subject population was relatively small in size and may not have presented the range of peritoneal conditions necessary for evaluating appropriately the role of effluent ADMA. Third, the serum ADMA level was not determined, which hindered the elucidation of the role of circulating ADMA.

CONCLUSION

Dialysis effluent ADMA had a positive correlation with effluent pentosidine in peritoneal dialysis patients, suggesting a possible intraperitoneal induction of ADMA by AGEs.

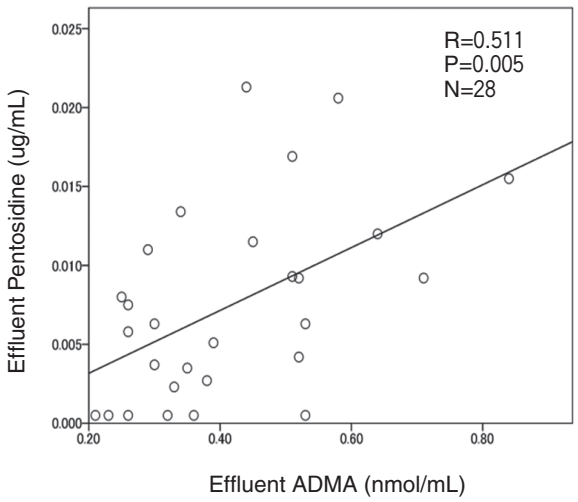

Figure Correlation between effluent asymmetric dimethylarginine and pentosidine in dialysis effluent of peritoneal dialysis patients
Dialysis effluent was collected after 8-h peritoneal dialysis from 28 patients. Concentrations of effluent asymmetric dimethylarginine (ADMA) and pentosidine were determined and plotted. Pearson's correlation coefficient was used for statistical analysis.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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