Original Paper

Effects of Acetate-Free Citrate Dialysate on Glycoxidation and Lipid Peroxidation Products in Hemodialysis Patients

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Abstract
Background/Aims: Previous studies have shown the presence of high levels of glycoxidation and lipid peroxidation products in association with atherosclerosis in patients with end-stage kidney disease. Acetates are commonly used buffer for correcting metabolic acidosis in hemodialysis (HD) patients. Since the toxic effects of acetates are well established, acetate-free citrate dialysate (AFD) has become available in Japan. The objective of the present study was to evaluate the suppressive effects of AFD on oxidative stress in maintenance HD patients by measuring plasma pentosidine and malondialdehyde-modified low-density lipoprotein (MDA-LDL) levels as markers for glycoxidation and lipid peroxidation products. ***Methods:*** Plasma pentosidine, MDA-LDL and other laboratory parameters were examined on maintenance HD at the Juntendo University Hospital before and after switching to AFD. ***Results:*** MDA-LDL levels divided by LDL cholesterol were significantly lower than those before switching to AFD. Furthermore, levels of plasma pentosidine were lower than those before switching to AFD. Stepwise multiple regression analysis revealed that the percent change of the calcium-phosphorus product in the nondiabetic group and that of phosphorus in the diabetic group were predictive variables for the percent change of MDA-LDL/LDL, whereas the percent change of log high-sensitive C-reactive protein and that of systolic blood pressure in the nondiabetic group and that of diastolic blood

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pressure in the diabetic group were predictive variables for the percent change of plasma pentosidine. **Conclusions:** It appears that AFD decreases glycoxidation and lipid peroxidation products when compared with acid citrate dextrose in HD patients. The reduction of oxidative stress by AFD during HD may have possible beneficial effects on atherosclerosis through calcium-phosphorus metabolism and blood pressure.

Introduction

Previous studies have shown that the presence of high levels of glycoxidation and lipid peroxidation products is associated with atherosclerosis, diabetes mellitus (DM) and aging [1–4]. Increased levels of these products have been well documented in patients with end-stage kidney disease (ESKD) [5, 6]. Sakata et al. [7] and Yamamoto et al. [8] demonstrated that glycoxidation and lipid peroxidation may promote the development of vascular complications, particularly atherosclerosis in patients with ESKD. Cardiovascular complications are the principal cause of morbidity and mortality in patients with ESKD who require hemodialysis (HD).

Plasma pentosidine is a major glycoxidation product formed under oxidative conditions. Several studies have demonstrated the increased concentrations of pentosidine in the tissues and plasma of uremic patients [9]. Malondialdehyde-modified low-density lipoprotein (MDA-LDL) is an end product of lipid peroxidation and may be involved in the early stages of atherosclerotic lesion progression. In addition, it is a useful biomarker of atherosclerosis [10, 11].

A conventional dialysate, an acetate-containing sodium bicarbonate solution, is currently used for standard HD in Japan. However, acetate-induced adverse effects such as arterial hypotension, depressed left ventricular function and increased incidences of nausea, vomiting and headache have been reported in numerous studies [12, 13]. Previous studies demonstrated that acetate promotes vascular damage and leads to the aforementioned adverse effects [12, 14].

Recently, acetate-free citrate dialysate (AFD) has become available in Japan. The objective of the present study in maintenance HD patients was to evaluate the beneficial effects of AFD on clinical parameters and to determine the attenuating effects of AFD on the glycoxidation and lipid peroxidation products by measuring plasma pentosidine and MDA-LDL levels.

Methods

Patient Characteristics
Fifty-nine patients on maintenance HD at the Juntendo University Hospital (39 males and 20 females; mean age 64 ± 1.7 years) participated in this study. The hospital ethics committee of the Juntendo University approved the study and informed consent was obtained from the patients enrolled in this study. The baseline characteristics of the patients are summarized in table 1. Their mean age was 64 ± 1.7 years and mean duration of HD therapy was 7.8 ± 0.8 years.

Dialysate
Before the study, acetate-containing dialysate was used in all patients at a flow rate of 500 ml/min from a central supply system, and then they were switched to AFD (Carbostar®,

Table 1. Baseline characteristics at the start of the study

Age, years	64 ± 1.7
Gender (male/female)	39/20
Duration of dialysis, years	7.8 ± 0.8
Body height, cm	160.5 ± 1.2
Body weight, kg	52.5 ± 1.5
Cause of kidney failure, n	
Glomerulonephritis	16
Diabetes mellitus	14
Nephrosclerosis	13
Polycystic kidney disease	5
Other	11

Ajinomoto Pharmaceuticals Co., Ltd., Tokyo, Japan). The composition of AFD was as follows: sodium 140 mEq/l, potassium 2.0 mEq/l, calcium 3.0 mEq/l, magnesium 1.0 mEq/l, chloride 111 mEq/l, bicarbonate 35 mEq/l and citrate 2.0 mEq/l. Conventional acid citrate dextrose composition was as follows: sodium 143 mEq/l, potassium 2.0 mEq/l, calcium 2.5 mEq/l, magnesium 1.0 mEq/l, chloride 112 mEq/l, bicarbonate 27.5 mEq/l (mmol/l) and acetate 11 mEq/l. With the exception of the amounts of erythropoiesis-stimulating agent and iron supplements, dialysis prescriptions did not change after switching to AFD.

Laboratory Parameters

Five blood samples were obtained from each patient, i.e. at the start of AFD (baseline) and at 1, 3, 6 and 12 months after switching to AFD. All samples were collected from the arterial line at the start of the HD session at the beginning of the week. Hemoglobin, hematocrit, serum total protein, serum albumin, serum lipids [LDL cholesterol, high-density lipoprotein (HDL) cholesterol, non-HDL cholesterol, and triglyceride] and high-sensitive C-reactive protein (hs-CRP) levels were measured using standard laboratory protocols of the Juntendo University Hospital. Plasma pentosidine and serum MDA-LDL levels were measured using enzyme-linked immunosorbent assays (plasma pentosidine, Fushimi Pharmaceutical, Kagawa, Japan; MDA-LDL, Sekisui Medical Co., Ltd., Tokyo, Japan). MDA-LDL levels divided by LDL cholesterol (MDA-LDL/LDL-C) were calculated as an indicator of the extent of LDL cholesterol oxidation. Moreover, the percent change of each parameter at 12 months after switching to AFD from baseline was calculated. Additionally, all patients were divided into a non-DM (NDM) group or a DM group, and the two groups were compared.

As a validated indicator of subclinical atherosclerosis and arterial stiffness, the measurements of carotid intima media thickness, ankle brachial pressure index and pulse wave velocity were examined at two points, namely, before and after switching to AFD using standard protocols of the Juntendo University Hospital. The measurements for each parameter were averaged to obtain the mean value.

Statistical Analysis

Data are expressed as means $\pm$ standard deviations. Statistical analyses were performed using Stat View software for Windows. Comparisons between more than two groups of patients were analyzed by one-way ANOVA with Fisher's test. First, correlation of the percent change of plasma pentosidine and MDA-LDL levels with that of other laboratory parameters was determined by single linear univariate analysis. Variables with a p value of <0.10 were then analyzed using a stepwise multivariate regression analysis on the basis of a forward-backward procedure. A logarithmic transformation of hs-CRP and triglyceride was used to

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Table 2. Blood pressure parameters

	At start	After			
		1 month	3 months	6 months	12 months
At beginning of dialysis					
Systolic BP, mm Hg	156 ± 3.5	152 ± 2.9	157 ± 2.6	153 ± 3.3	153 ± 3.1
Diastolic BP, mm Hg	78 ± 2.1	76 ± 1.8	78 ± 1.4	77 ± 1.7	76 ± 1.5
Pulse pressure, mm Hg	79.4 ± 2.3	77.7 ± 2.2	80.0 ± 2.1	76.6 ± 2.6	78.3 ± 2.5
Mean BP, mm Hg	104.0 ± 2.3	101.4 ± 1.9	104.4 ± 1.6	102.3 ± 2.0	102.3 ± 1.8
At completion of dialysis					
Systolic BP, mm Hg	148 ± 3.3	145 ± 3.4	145 ± 3.1	142 ± 3.0	149 ± 3.2
Diastolic BP, mm Hg	78 ± 1.7	77 ± 2.0	77 ± 1.7	75 ± 1.6*	75 ± 1.7
Pulse pressure, mm Hg	70.7 ± 2.6	68.7 ± 2.4	69.5 ± 2.4	68.4 ± 2.6	75.0 ± 3.0
Mean BP, mm Hg	101.9 ± 1.9	100.0 ± 2.3	99.9 ± 1.9	97.5 ± 1.8*	100.2 ± 1.7

BP = Blood pressure. * $p < 0.05$ vs. at start.

Fig. 1. Change in systolic blood pressure during HD sessions [Δ systolic blood pressure = (systolic blood pressure at the completion of HD) – (systolic blood pressure at the start of HD)]. No significant differences were observed during the study.
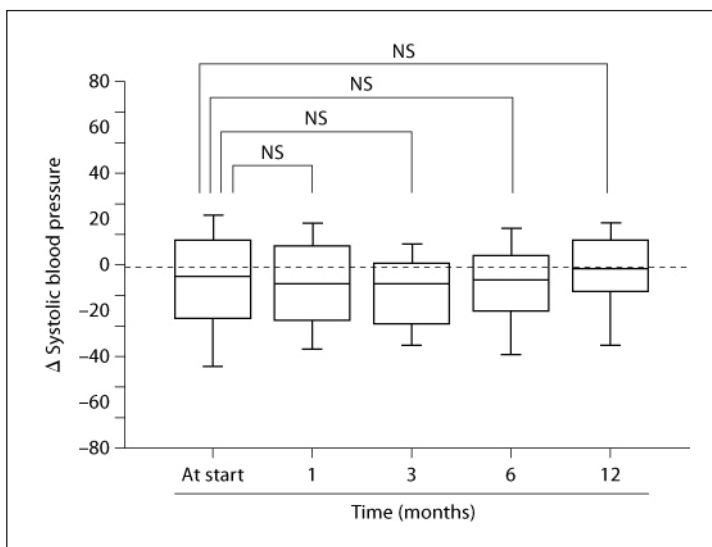
render the normal distribution for parametric tests. The F value for entry or removal of candidate variables from discriminant function was set at 4.0. A p value <0.05 was defined as statistically significant.

Results

Blood Pressure

The blood pressure data are shown in table 2. There were no significant differences in systolic and diastolic blood pressures, pulse pressure and mean blood pressure at the beginning of the dialysis session and after switching to AFD. Figure 1 shows changes in the systolic blood pressure gap, defined as post-dialysis systolic pressure minus pre-dialysis systolic pressure for a single HD session. There was no statistically significant change in the

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Table 3. Biochemical parameters

	At start	After			
		1 month	3 months	6 months	12 months
Creatinine, mg/dl	11.2 ± 0.3	11.3 ± 0.3	11.2 ± 0.3	11.1 ± 0.3	11.1 ± 0.3
SUN, mg/dl	66.5 ± 1.9	67.3 ± 2.0	68.0 ± 2.3	66.7 ± 2.2	61.2 ± 2.1*
Non-HDL cholesterol, mg/dl	108.1 ± 4.3	115.0 ± 5.4	124.0 ± 6.8	119.1 ± 4.5	120.4 ± 4.1*
TG, mg/dl	100.4 ± 9.4	104.9 ± 9.8	104.3 ± 9.5	125.1 ± 12.8*	117.4 ± 11.9
HDL cholesterol, mg/dl	45.8 ± 1.7	45.0 ± 2.5	45.3 ± 2.1	47.3 ± 2.0	47.5 ± 1.6
LDL cholesterol, mg/dl	79.2 ± 3.2	87.3 ± 4.7	92.2 ± 4.2*	85.3 ± 3.7	89.8 ± 3.8*
Total protein, g/dl	6.5 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.6 ± 0.1*	6.6 ± 0.1
Albumin, g/dl	3.9 ± 0.1	3.8 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.1
hs-CRP, mg/dl	0.336 ± 0.174	0.466 ± 0.221	0.410 ± 0.242	0.409 ± 0.170	0.406 ± 0.208
Sodium, mEq/l	141 ± 0.4	139 ± 0.3**	139 ± 0.4**	139 ± 0.3*	140 ± 0.4*
Potassium, mEq/l	5.1 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.1 ± 0.1	5.0 ± 0.1
Corrected calcium, mg/dl	9.4 ± 0.1	9.4 ± 0.1	9.5 ± 0.1	9.5 ± 0.1	9.6 ± 0.1*
Phosphorus, mg/dl	5.2 ± 0.2	5.0 ± 0.2	5.2 ± 0.2	5.4 ± 0.2	4.9 ± 0.1*
Ca × P, mg²/dl²	48.8 ± 1.61	47.5 ± 1.52	48.9 ± 1.45	51.2 ± 1.50	46.9 ± 1.06
Kt/V urea	1.39 ± 0.03	1.42 ± 0.03	1.38 ± 0.04	1.40 ± 0.03	1.37 ± 0.03

Non-HDL cholesterol, LDL cholesterol and HDL cholesterol were analyzed by the two-sided t test.
SUN = Serum urea nitrogen; TG = triglyceride.
* $p < 0.05$ vs. at start. ** $p < 0.0001$ vs. at start.

levels of the systolic pressure gap after switching to AFD. Moreover, the diastolic blood pressure gap during the HD session did not change with AFD (data not shown).

Biological Parameters

The biological parameters are listed in table 3. The level of plasma triglyceride at 6 months after switching to AFD was significantly increased from 100.4 ± 9.4 to 125.1 ± 12.8 mg/dl ($p < 0.05$). However, no significant change was observed at 12 months after switching to AFD. Non-HDL cholesterol level at 12 months after switching to AFD was significantly increased from 108.1 ± 4.3 to 120.4 ± 4.1 mg/dl ($p < 0.05$). In addition, LDL cholesterol levels at 3 and 6 months after switching to AFD were significantly increased from 79.2 ± 3.2 to 92.2 ± 4.2 mg/dl ($p < 0.05$) and then to 89.3 ± 3.8 mg/dl ($p < 0.05$). Levels of plasma sodium were significantly decreased after switching to AFD (baseline, 141.0 ± 0.4 mEq/l; 1 month, 139.0 ± 0.3 mEq/l ($p < 0.001$); 3 months, 139.0 ± 0.4 mEq/l ($p < 0.001$); 6 months, 139.0 ± 0.3 mEq/l ($p < 0.05$), and 12 months, 140.0 ± 0.4 mEq/l ($p < 0.05$). The level of serum urea nitrogen at 12 months after switching to AFD was significantly decreased from 66.5 ± 1.9 to 61.2 ± 2.1 mg/dl ($p < 0.05$). The levels of corrected calcium were significantly increased and those of phosphorus significantly decreased at 12 months after switching to AFD. There were no significant changes in the levels of serum creatinine, albumin, HDL cholesterol, hs-CRP or potassium levels after switching to AFD. The efficacy of dialysis calculated by Kt/V was evaluated, although no significant change in efficacy after switching to AFD was observed. Compared with NDM patients, DM patients had higher plasma triglyceride but lower hs-CRP at 12 months after switching to AFD, but there were no significant changes in the percent change of each parameter (table 4).

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Table 4. Comparison of the characteristics between nondiabetic and diabetic HD patients

	At start		p value	After 12 months		p value	% change from baseline		p value
	nondiabetic	diabetic		nondiabetic	diabetic		nondiabetic	diabetic	
MDA-LDL/LDL (U·dl/mg·l)	1.232 ± 0.055	1.324 ± 0.083	0.3997	1.005 ± 0.045	0.980 ± 0.079	0.7974	−11.3 ± 5.7	−16.7 ± 9.5	0.6692
Pentosidine, μg/dl	0.539 ± 0.031	0.461 ± 0.075	0.2620	0.369 ± 0.025	0.300 ± 0.051	0.2136	−29.4 ± 2.6	17.6 ± 9.2	0.0913
TG, mg/dl	98.7 ± 7.6	116.6 ± 17.1	0.2805	102.2 ± 9.0	162.7 ± 25.1	0.0072	13.2 ± 9.2	52.3 ± 23.9	0.0754
Albumin, g/dl	3.8 ± 0.1	4.0 ± 0.1	0.1125	3.9 ± 0.1	4.1 ± 0.1	0.0703	1.5 ± 1.1	1.0 ± 1.5	0.8533
hs-CRP, mg/dl	0.387 ± 0.196	0.385 ± 0.175	0.9965	0.480 ± 0.258	0.097 ± 0.061	0.4490	98.6 ± 56.2	−19.8 ± 18.5	0.2851
Corrected calcium, mg/dl	9.5 ± 0.1	9.3 ± 0.1	0.2873	9.7 ± 0.1	9.1 ± 0.3	0.0134	29.2 ± 10.4	−9.0 ± 23.1	0.1075
Phosphorus, mg/dl	5.3 ± 0.2	5.1 ± 0.4	0.7594	4.9 ± 0.1	4.7 ± 0.3	0.4095	−2.4 ± 3.1	−6.2 ± 6.6	0.5894
Ca × P, mg²/dl²	49.6 ± 1.7	47.7 ± 3.2	0.5787	47.9 ± 1.0	43.2 ± 3.0	0.0703	0.6 ± 3.2	−6.5 ± 8.1	0.3412
PH	7.368 ± 0.006	7.380 ± 0.006	0.2788	7.371 ± 0.007	7.376 ± 0.008	0.7163	0.008 ± 0.075	−0.05 ± 0.103	0.7030
HCO₃⁻, mmol/l	22.3 ± 0.6	22.4 ± 0.5	0.9056	22.4 ± 0.5	21.8 ± 0.6	0.5292	−0.1 ± 2.5	−3.6 ± 3.9	0.4947
Systolic BP, mm Hg	152.4 ± 3.4	158.0 ± 8.3	0.4606	151.7 ± 3.6	158.8 ± 6.0	0.3449	−0.2 ± 2.3	−0.5 ± 5.6	0.9616
Diastolic BP, mm Hg	75.2 ± 1.9	76.5 ± 5.8	0.7824	74.8 ± 1.7	79.9 ± 3.3	0.1601	0.1 ± 2.2	−1.3 ± 5.2	0.7630
Hemoglobin, g/dl	10.5 ± 0.1	10.5 ± 0.3	0.9927	10.6 ± 0.2	11.2 ± 0.3	0.1085	−0.3 ± 1.6	5.6 ± 2.7	0.0846
Hematocrit, %	32.8 ± 0.4	32.4 ± 1.0	0.5881	32.7 ± 0.5	34.0 ± 0.9	0.2398	−0.6 ± 1.5	5.9 ± 3.0	0.0507
IMT, mm	0.87 ± 0.03	0.92 ± 0.05	0.3568	0.86 ± 0.02	0.90 ± 0.03	0.2755	−0.1 ± 1.9	−0.1 ± 4.2	0.9904
PWV, cm/s	1,741 ± 55	1,742 ± 148	0.9868	1,751 ± 65	1,676 ± 99	0.5675	1.3 ± 2.5	4.1 ± 3.7	0.5759
ABI	1.22 ± 0.03	1.14 ± 0.07	0.4670	1.22 ± 0.03	1.18 ± 0.08	0.6125	3.1 ± 2.4	−0.3 ± 2.9	0.4678

TG = Triglyceride; BP = blood pressure; IMT = intima media thickness; ABI = ankle brachial pressure index; PWV = pulse wave velocity.

Table 5. Data on arterial blood gas

	At start	After			
		1 month	3 months	6 months	12 months
pH	7.37 ± 0.01	7.38 ± 0.01	7.37 ± 0.01	7.39 ± 0.00*	7.34 ± 0.01
PCO₂, mm Hg	40.0 ± 0.8	40.0 ± 0.5	40.4 ± 0.6	39.2 ± 0.5	38.9 ± 0.6
PO₂, mm Hg	101.0 ± 3.2	99.9 ± 3.0	106.0 ± 2.6	103.2 ± 3.0	94.2 ± 2.7*
HCO₃⁻, mmol/l	22.7 ± 0.5	22.9 ± 0.4	23.0 ± 0.4	23.1 ± 0.4	22.2 ± 0.4
Anion gap	13.8 ± 0.5	13.3 ± 2.7	15.7 ± 0.4	15.1 ± 0.5	16.3 ± 0.5

* p < 0.05 vs. at start.

Acidosis

The pH level at the beginning of the HD session at 6 months after switching to AFD was significantly increased from 7.372 ± 0.005 to 7.387 ± 0.005 (p < 0.05). However, there was no significant difference between the pH levels at baseline and at 12 months (table 5). There was no significant change in bicarbonate levels after switching to AFD. Other parameters also did not change significantly.

Management of Renal Anemia and Metabolism of Iron

As shown in tables 6 and 7, the new dialysate did not affect the levels of hematocrit, hemoglobin, iron and ferritin, or the dose of recombinant human erythropoietin and iron supplements with the exception of darbepoetin alfa dosage, which was increased from 80.0 ± 15.1 to 101.7 ± 17.8 U/month and then to 113.9 ± 19.7 U/month at 6 and 12 months after switching to AFD. The total iron binding capacity at 6 months after switching to AFD was increased from 252 ± 6.5 to 264 ± 6.3 μg/dl.

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Table 6. Parameters assessing hemoglobin, hematocrit and iron stores

	At start	After			
		1 month	3 months	6 months	12 months
Hemoglobin, g/dl	10.5 ± 0.1	10.4 ± 0.1	10.6 ± 0.1	10.6 ± 0.2	10.7 ± 0.1
Hematocrit, %	32.8 ± 0.4	32.2 ± 0.5	33.1 ± 0.5	33.0 ± 0.5	33.0 ± 0.4
Iron, μg/dl	60.8 ± 3.0	64.3 ± 3.5	61.5 ± 3.2	57.8 ± 3.3	61.6 ± 3.7
TIBC, μg/dl	252 ± 6.5	252 ± 6.0	255 ± 5.8	264 ± 6.3*	258 ± 5.7
Ferritin, ng/ml	174 ± 21.9	164 ± 20.5	160 ± 20.2	174 ± 26.5	142 ± 19.8

* $p < 0.05$ vs. at start. TIBC = Total iron-binding capacity.

Table 7. Data on erythropoiesis-stimulating agents and intravenous iron supplement dosage

	At start	After			
		1 month	3 months	6 months	12 months
rHuEPO dosage, U/month	18,666 ± 4,494	19,000 ± 4,416	20,000 ± 3,708	24,000 ± 3,969	23,111 ± 4,712
Darbepoetin alfa dosage, U/month	80.0 ± 15.1	88.3 ± 18.1	86.4 ± 16.3	101.7 ± 17.8*	113.9 ± 19.7*
Iron supplement dosage, mg/month	60.0 ± 11.3	43.3 ± 10.4	56.7 ± 11.2	36.7 ± 10.9	36.7 ± 9.8

Three patients did not take any erythropoiesis-stimulating agents (other patients took rHuEPO or darbepoetin alfa). rHuEPO = Recombinant human erythropoietin.

* $p < 0.05$ vs. at start.

Markers of Glycoxidation and Lipid Peroxidation Products

Plasma pentosidine levels at 3, 6 and 12 months after switching to AFD were significantly decreased from 0.505 ± 0.031 to 0.415 ± 0.026 μg/ml ($p < 0.0001$), to 0.449 ± 0.028 μg/ml ($p < 0.0001$) and then to 0.358 ± 0.023 μg/ml ($p < 0.0001$) (fig. 2). Furthermore, MDA-LDL/LDL-C levels at 6 and 12 months after switching to AFD were significantly decreased from 1.254 ± 0.046 to 1.075 ± 0.065 U · dl/mg · l ($p < 0.05$) and then to 1.000 ± 0.039 U · dl/mg · l ($p < 0.0001$) (fig. 3).

Correlation between Glycoxidation or Lipid Peroxidation Products and Each Parameter

Single linear univariate analysis (table 8) showed that the percent change of MDA-LDL/LDL was correlated positively with that of phosphorus ($r = 0.339$, $p = 0.0401$) and the calcium-phosphorus product (Ca × P; $r = 0.336$, $p = 0.0261$). The percent change of plasma pentosidine was correlated negatively with that of log hs-CRP ($r = -0.351$, $p = 0.0359$) and positively with that of systolic blood pressure ($r = 0.390$, $p = 0.0186$) in the nondiabetic group. In the diabetic group, the percent change of MDA-LDL/LDL was correlated positively with that of phosphorus ($r = 0.663$, $p = 0.0516$) and Ca × P ($r = 0.640$, $p = 0.0637$). The percent change of plasma pentosidine was correlated negatively with that of systolic blood pressure ($r = -0.597$, $p = 0.0687$) and diastolic blood pressure ($r = -0.7171$, $p = 0.0197$). Stepwise multiple regression analysis revealed that the percent change of Ca × P in the nondiabetic group and that of phosphorus in the diabetic group were predictive variables for the percent change of MDA-LDL/LDL, whereas the percent change of log hs-CRP and that of systolic blood pressure in the nondiabetic group and that of diastolic blood pressure in the diabetic group were predictive variables for the percent change of plasma pentosidine (table 9).

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Fig. 2. Plasma pentosidine levels decreased significantly from 0.505 ± 0.031 to 0.415 ± 0.026 µg/ml at 3 months, 0.449 ± 0.028 µg/ml at 6 months, and 0.358 ± 0.023 µg/ml at 12 months. NS = Not significant.
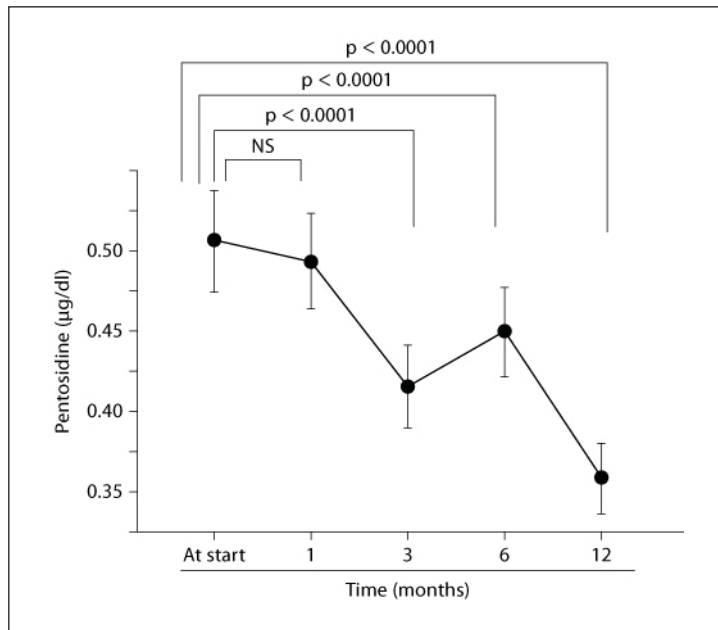
Fig. 3. Serum MDA-LDL/LDL-C levels decreased significantly from 1.254 ± 0.046 to 1.075 ± 0.065 U · dl/mg · l (48.5 ± 1.78 to 41.6 ± 2.51 U/mmol) at 6 months and then to 1.000 ± 0.039 U · dl/mg · l (38.7 ± 1.51 U/mmol) at 12 months. NS = Not significant.
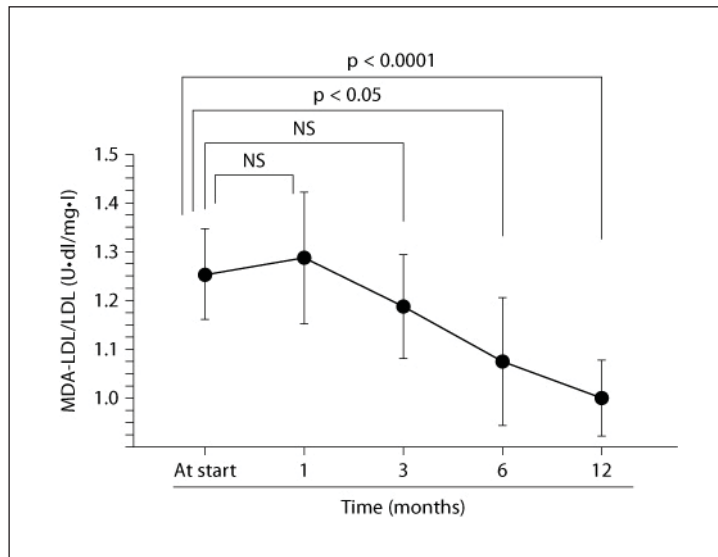
Discussion

In this study, we clarified the clinical effects of the removal of acetate from dialysate using an AFD that was developed in Japan. Until recently, acetates have been used for many years as buffer in conventional dialysates. However, it is well known that acetates lead to acetate intolerance such as arterial hypotension, nausea, vomiting, headache and decreased left ventricular function [15]. Furthermore, Stenvinkel et al. [16] reported that HD patients often experience malnutrition, inflammation, atherosclerosis (MIA syndrome) and increased oxidative stress.

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Table 8. Single univariate linear correlation of percent change of factors associated with percent change of MDA-LDL/LDL-C and pentosidine levels in nondiabetic and diabetic groups

	Nondiabetic				Diabetic			
	MDA-LDL/LDL-C		pentosidine		MDA-LDL/LDL-C		pentosidine	
	r	p value	r	p value	r	p value	r	p value
log TG	−0.151	0.3711	0.140	0.4139	−0.011	0.9778	−0.348	0.3244
Albumin	0.155	0.3587	0.183	0.2849	0.509	0.1614	−0.051	0.8894
log hs-CRP	0.067	0.6926	−0.351	0.0359	0.295	0.4413	−0.478	0.1625
Corrected calcium	0.045	0.7893	−0.034	0.8452	0.236	0.5405	−0.199	0.5818
Phosphorus	0.339	0.0401	−0.020	0.9101	0.663	0.0516	0.256	0.4754
Ca $\times$ P	0.336	0.0261	−0.026	0.8808	0.640	0.0637	0.165	0.6479
PH	−0.057	0.7469	0.064	0.7193	−0.067	0.8642	−0.229	0.5249
HCO_3^-	−0.059	0.7341	−0.055	0.7531	−0.149	0.7014	0.113	0.7556
Systolic BP	0.161	0.3421	0.390	0.0186	0.134	0.7308	−0.597	0.0687
Diastolic BP	0.107	0.5362	−0.013	0.9390	−0.171	0.6609	−0.717	0.0197
Hemoglobin	−0.246	0.1428	0.012	0.7772	−0.249	0.5185	−0.377	0.2825
Hematocrit	−0.223	0.1849	0.012	0.9448	−0.360	0.3411	−0.443	0.2001
IMT	−0.038	0.8356	0.032	0.8639	−0.326	0.3926	0.120	0.7415
PWV	0.201	0.3060	0.057	0.7758	0.552	0.1986	−0.419	0.3017
ABI	0.164	0.4125	0.265	0.1903	0.407	0.3653	−0.188	0.6550

BP = Blood pressure; TG = triglyceride.

Table 9. Stepwise multiple regression analysis of factors associated with percent change of MDA-LDL/LDL and pentosidine in nondiabetic and diabetic groups

	B values	F values
Nondiabetic		
MDA-LDA/LDL		
Phosphorus	−0.021	0.015
Ca $\times$ P	0.366	5.398
Pentosidine		
log hs-CRP	−0.353	5.677
Systolic BP	0.392	7.015
Diabetic		
MDA-LDA/LDL		
Phosphorus	0.663	5.489
Ca $\times$ P	−0.013	0.001
Pentosidine		
Diastolic BP	−0.717	8.455
Systolic BP	0.145	0.15

BP = Blood pressure.

This study demonstrated the suppressive effects of AFD on glycoxidation and lipid per-oxidation products in HD patients. Acetate is considered to be one of the bioincompatible factors along with the purity and quality of the dialysis solutions or dialysis membrane type. Use of this bioincompatible dialysis system results in the increased production of free radical or reactive oxygen species (ROS). Todeschini et al. [17] reported limited elevation in super-oxide production by neutrophils during acetate-free biofiltration compared to bicarbonate

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dialysis. Carozzi et al. [18] reported that acetate may induce monocyte activation and cytokine production. Furthermore, Higuchi et al. [19] reported that cytokine production was minimal during acetate-free biofiltration. In view of these findings, we hypothesize that absence of acetate may contribute to the decreases in plasma pentosidine and MDA-LDL/LDL which are indicators of glycoxidation and lipid peroxidation products in the presence of oxidative stress.

Our results indicate that AFD reduced the production of oxygen free radicals and inflammatory cytokines, which in turn contributed to a possible improvement in the progression of atherosclerosis. Various studies have reported that the increases in oxidative stress and micro-inflammation are associated with increased atherosclerosis, cardiovascular morbidity and mortality [20–22]. Zhou et al. [23] reported that serum pentosidine was associated with arterial stiffness in HD patients. Generally, DM is characterized by higher levels of oxidative stress. The existence of increased oxidative stress is based on decreased antioxidant capacity in DM along with chronic exposure to increased levels of ROS and increased glycoxidation and peroxidation. Nevertheless, plasma pentosidine, MDA-LDL/LDL levels and other laboratory parameters were not significantly changed in patients with or without DM in this study. Previous studies demonstrated similar results in that no significant difference was found in serum pentosidine level, a marker of glycoxidation, in ESKD patients with or without DM [24]. We consider that diabetic patients receiving HD are consistently exposed to not only hyperglycemia but also uremic status, malnutrition, micro-inflammation and aging which causes oxidative stress in comparison to diabetic patients without renal complications.

By the stepwise multiple regression analysis (table 9), our study demonstrated a correlation between the percent change of plasma pentosidine and that of blood pressure and hs-CRP, and between the percent change of MDA-LDL/LDL and that of calcium-phosphorus metabolism in patients with or without DM. Several studies have described the relationship between elevation of calcium-phosphorus metabolism and vascular calcification or high-mortality risk in ESKD patients [25–27]. Dalfino et al. [28] reported that oxidative stress could increase the secretion of bone morphogenetic protein-2, a member of the transforming growth factor-β superfamily. Furthermore, they showed that increase in bone morphogenetic protein-2 had a direct effect on vascular smooth muscle cells and regulated vascular calcification through increased phosphorus uptake. Another study demonstrated that elevation of phosphorus levels causes endothelial dysfunction and inhibition of nitric oxide (NO) production. This occurred through increased ROS production and endothelial NO synthase inactivation via protein kinase C, resulting in impaired endothelium-dependent vasodilation [29]. Thus, our result shows that the reduction of oxidative stress by AFD during HD may have possible beneficial effects on atherosclerosis through calcium-phosphorus metabolism and blood pressure.

Moreover, we investigated data of carotid intima media thickness, pulse wave velocity and ankle brachial pressure index, as indicators of atherosclerosis and arterial stiffness but no significant differences were observed. This may be due to a short 12-month study period and to the fact that such long-term observation is required to examine the effects on atherosclerosis and subsequent mortality.

Blood analysis showed that the pH, bicarbonate and anion gap levels were similar before and after switching to AFD. This finding indicates that acetate-free citrate-buffered dialysis fluids correct metabolic acidosis safely following proper replacement of the acetate-containing dialysate. Biochemical parameters such as corrected calcium increased significantly, and phosphorus levels decreased significantly at 12 months after replacement with AFD. In this study, the new dialysate concentration of calcium was increased from 2.5 to 3.0 mEq/l, and this change is considered reasonable and appropriate. No other obvious side effects or adverse events were observed with switching to AFD.

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We observed no significant differences in the intradialytic blood pressure measured throughout the study. However, several reports showed that acetate-free renal replacement therapy had beneficial effects on intradialytic hypotension compared with acetate-containing therapies [30, 31]. Acetates may induce the production of cytokines and NO, a vasodilator that can cause intradialytic cardiovascular instability [12, 30]. Possible reasons for the different finding may be that there were few previous episodes of intradialytic hypotension, and blood pressure was well-controlled in our patient group. Alternatively, it is possible that our data may have been influenced by factors other than dialysate composition, such as administration of anti-hypertensive drugs, differences in dry weight or seasonal variations. It was therefore difficult to objectively interpret our findings.

Beneficial effects of AFD on anemia associated with renal replacement therapy have been reported in several studies [31, 32]. However, our data provides conflicting results from previous reports, since the erythropoiesis-stimulating agent requirement tended to rise, while the intravenous dosage of iron supplement decreased. It was proposed that beneficial effects of AFD on anemia were attributable to the addition of citrate, which has a local anti-coagulation effect that inhibits clotting. Maintenance of an effective membrane surface area throughout dialysis may lead to an increase in dialysis efficacy [33]. Other reasons why acetates cause oxidative stress and micro-inflammation, which induces malnutrition and anemia, have also been reported [32]. The precise reasons for the discrepancy between our results and those of earlier studies are unknown. In this study, the type of dialyser, duration of dialysis, dialysate and blood flow remained unchanged throughout the study. The baseline state of anemia was well-controlled in our study group, but it is possible that potential iron deficiency may have affected our results.

Limitations of this study were that the investigation was single arm and not randomized. The treatment duration was relatively short. Furthermore, the majority of participant conditions were well-controlled in terms of anemia, nutrition status, blood pressure and balance of body fluids. A multi-center, randomized trial should be considered in the future.

It appears that HD treatment with AFD decreases glycoxidation and lipid peroxidation products compared with conventional acetate-containing dialysate. The reduction of oxidative stress by AFD during HD may have possible beneficial effects on atherosclerosis through calcium-phosphorus metabolism or blood pressure.

Acknowledgement

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