Hemodialysis International 2013; ••:••–••

Effect of a hydrogen (H_2)-enriched solution on the albumin redox of hemodialysis patients

Hiroyuki TERAWAKI,[1] Wan-Jun ZHU,[2] Yukie MATSUYAMA,[3] Tomoyoshi TERADA,[3] Yasuhito TAKAHASHI,[1] Kaoru SAKURAI,[1] Shigeru KABAYAMA,[2] Mariko MIYAZAKI,[2] Noritomo ITAMI,[4] Ryoichi NAKAZAWA,[5] Sadayoshi ITO,[2] Seiichi ERA,[3] Masaaki NAKAYAMA[1,2]

[1]Dialysis Center, Fukushima Medical University, Fukushima, Japan; [2]Center for Advanced and Integrated Renal Science, Tohoku University Graduate School of Medicine, Sendai, Japan; [3]Department of Physiology and Biophysics, Gifu University Graduate School of Medicine, Gifu, Japan; [4]Nikko Memorial Hospital, Muroran, Japan; [5]Mito Chuo Clinic, Mito, Japan

Abstract

Elevated oxidative stress (OS) is associated with severe cardiovascular disease and premature death among patients treated with hemodialysis (HD). Oxidative stress is enhanced by contact between blood and dialysis membranes during HD sessions. This study aimed to clarify whether hydrogen (H_2), which is a known antioxidant, is capable of suppressing increased OS induced during HD sessions. Eight patients on regular HD treatment were studied. Two HD sessions were performed in a cross-over design trial using standard and hydrogen-enriched solutions (mean of 50 p.p.b. H_2; H_2-HD). Blood samples were obtained from the inlet and outlet of the dialyzer during HD to determine changes in plasma levels of glutathione, hydrogen peroxide, and albumin redox state as a marker of OS. Comparison of inlet and outlet blood revealed significant decreases in total glutathione and reduced glutathione, as well as significant increases in hydrogen peroxide in both HD treatments. However, the mean proportion of reversibly oxidized albumin in outlet serum was significantly lower than that in inlet serum following the H_2-HD session, whereas no significant changes were found in the standard solution session, suggesting that "intra-dialyzer" OS is reduced by H_2-HD. In conclusion, the application of H_2-enriched solutions could ameliorate OS during HD.

Key words: Molecular hydrogen, oxidative stress, albumin redox state, hemodialysis

INTRODUCTION

Oxidative stress (OS) is a critical pathology that is a causative factor for cardiovascular diseases (CVDs) and premature death among patients with chronic kidney disease (CKD) and those on chronic dialysis therapy.[1,2] In fact, elevated OS characterized by enhanced albumin oxidation is strongly correlated with CVD incidence among CKD patients treated with hemodialysis (HD)[3] and peritoneal dialysis.[4]

There are multiple factors that can contribute to elevated OS in patients on HD therapy including the enhancement of pro-oxidants, which include accumulation of uremic oxidants,[5–11] excessive neutrophil

Correspondence to: H. Terawaki, MD, PhD, Dialysis Center, Fukushima Medical University, 1 Hikariga-oka, Fukushima 960-1285, Japan. E-mail: terawaki@fmu.ac.jp
Conflict of interest: None.
Disclosure of grants or other funding: None.

DOI:10.1111/hdi.12112

respiratory bursting and myeloperoxidase (MPO) release,[12,13] activation of monocytes to produce proinflammatory molecules during HD,[14] and the attenuation of antioxidants.[15] Therefore, the development of antioxidant therapy for HD patients is a recognized priority.

Recently, it has been shown that the hydrogen molecule (H_2) has a unique biological capacity to act as an antioxidative and anti-inflammatory substance.[16] Accumulating evidence indicates that H_2 administration ameliorates organ damage in various models of ischemia and inflammation.[17] For this reason, clinical applications of H_2 in proinflammatory disorders are being actively investigated, particularly in HD therapy by delivering H_2-enriched HD solutions (mean of 50 p.p.b.).[18–20] To date, the potential clinical merits of this strategy have been reported, such as decreased plasma MPO, monocyte chemoattractant protein 1 (MCP-1), and improvements in blood pressure control.[20]

The aim of this study is to examine alterations in the redox state of serum albumin, the marker of OS, in order to clarify the potential benefit of H_2-enriched HD solutions in ameliorating OS during HD treatment.

METHODS

Patients

Eight stable patients treated with regular HD (3 men and 5 women, mean age of 58 ± 16 years, mean duration of HD 111 ± 93 months) at Fukushima Medical University (Fukushima, Japan) were enrolled into the study between November and December 2012.

All patients were on standard bicarbonate HD (4 hours three times weekly) using hollow-fiber, high-flux biocompatible membrane dialyzers. Patients' characteristics are shown in Table 1. None of the patients had received antioxidant agents, such as vitamin C or E, and all prescribed medications had been unchanged throughout this study. Patients with infections, bleeding, liver dysfunction, collagen disease, or systemic vasculitis were not included in the present study.

Table 1 Patients' characteristics

Age (y)	58 ± 16
Gender (male : female)	3:5
Dialysis duration (mo)	93 ± 33
Primary disease	
Diabetic nephropathy	3
Chronic glomerulonephritis	3
Hypertensive kidney disease	2
Dialysis membrane	
Polysulfone	6
Ethylene vinyl alcohol copolymer	1
Triacetylcellulose	1

In this cross-over study, participants were first treated with HD using either hydrogen-enriched dialysate (H_2-HD) or standard dialysate (S-HD) in a midweek session (Wednesday or Thursday), and subsequently with HD using the opposite dialysate in a weekend session (Friday or Saturday). The order of dialysate use was assigned randomly using a random number table.

Blood samples were obtained from the inlet and outlet of the dialysis apparatus at the start (pre; 5 minutes after start) and end of HD (post; 5 minutes before end) (see Figure 1). For each blood sample, human serum albumin (HSA)-redox and levels of H_2, reduced glutathione (rGSH), total glutathione (tGSH), and hydrogen peroxide (H_2O_2) were measured. Blood samples for HSA-redox measurement were stored at –80°C for 2–4 weeks until analysis. Similarly, dialysate samples were obtained from the inlet and outlet of the dialysis apparatus at the start (pre) and end (post) of HD, and levels of H_2, pH, pCO_2, and pO_2 were measured.

This study was performed according to the principles of the Declaration of Helsinki and was approved by the local ethics committee (Fukushima Medical University, accept no. 1535). All subjects provided written informed consent.

H_2-HD system

Details of the system used for generating H_2-enriched solution have been reported previously.[19] Briefly, prefiltered water was processed using activated charcoal filtration and water softening to supply the water electrolysis system (HD-24K; Nihon Trim, Osaka, Japan), where water was electrolyzed by direct current supply to the anode and cathode electrode plates. Water on the anode side was drained out, and water from the cathode side (which contains highly dissolved H_2) was collected to supply the reverse osmosis equipment (MH1000CX; Japan Water System, Tokyo, Japan) at 500 mL/min. The intensity of electrolysis was adjusted to maintain pH 10.0. The reverse osmosis water made by the water electrolysis system was supplied to a personal HD monitoring system (DBB-27 or DBG-02; Nikkiso, Tokyo, Japan) to make the HD solution by mixing with a liquid solution concentrate (Kindaly AF-2; Fuso Pharm. Ind., Osaka, Japan). High pH of the cathode-side water was buffered by ingredients of the solution concentrate (mainly bicarbonate), and as a result, final pH of H_2-enriched solution and standard solution was similar (Table 2).

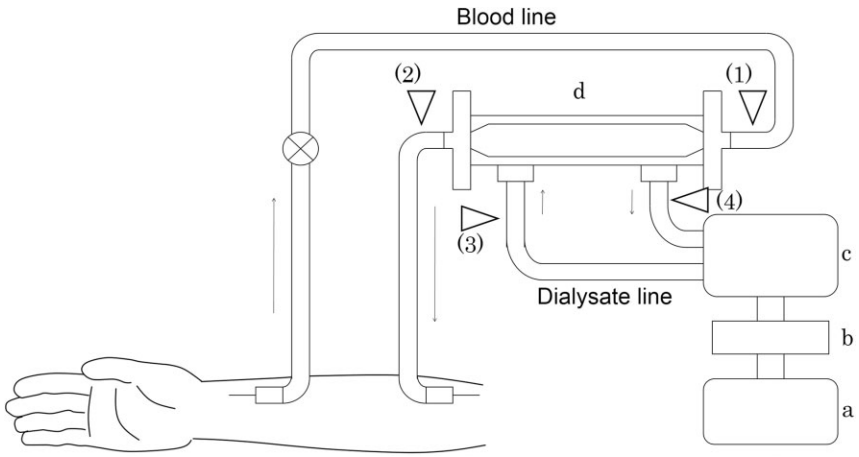

Figure 1 Schematic of the hemodialysis system. Blood samples were obtained from the inlet (1) and outlet (2) of the dialysis apparatus. Dialysate samples were also obtained from the inlet (3) and outlet (4) of the dialysis apparatus: (a) reverse osmosis (RO) system; (b) endotoxin cut-off filter; (c) dialysis machine; (d) dialysis apparatus (dialyzer).

Measurements

Plasma H_2O_2 concentrations were determined using a fluorescence spectrometric assay (Amplex Red hydrogen peroxide assay kit, Molecular Probes, Eugene, OR, USA) as described previously.[21,22] Plasma rGSH and tGSH concentrations were determined using a glutathione assay kit (BioVision Research Products, Mountain View, CA, USA) according to the manufacturer's recommendations. Dialysate pH, pCO_2 and pO_2 were determined using standard laboratory techniques.

Table 2 Mean pH, pCO_2, and pO_2 of each dialysate

		S-HD	H_2-HD
pH			
Pre	Inlet	7.42 ± 0.05	7.39 ± 0.08
	Outlet	7.37 ± 0.04	7.39 ± 0.05
Post	Inlet	7.42 ± 0.05	7.46 ± 0.05
	Outlet	7.31 ± 0.03	7.38 ± 0.04
pCO_2 (mmHg)			
Pre	Inlet	37.2 ± 3.6	42.1 ± 9.2
	Outlet	44.8 ± 3.0	41.8 ± 5.5
Post	Inlet	39.7 ± 6.6	36.8 ± 4.1
	Outlet	43.5 ± 3.5	42.4 ± 5.1
pO_2 (mmHg)			
Pre	Inlet	124.1 ± 14.3	133.5 ± 16.1
	Outlet	137.9 ± 9.6	137.5 ± 21.0
Post	Inlet	128.5 ± 19.4	127.6 ± 13.1
	Outlet	130.8 ± 24.9	131.5 ± 13.9

H_2-HD = hemodialysis using hydrogen-enriched dialysate; S-HD = hemodialysis using standard dialysate.

Levels of H_2 were determined using the gas chromatograph with a semiconductor detector (TRIlyzer mBA-3000, Taiyo Instruments Co., Osaka, Japan) according to the manufacturer's instruction. Briefly, samples (50 μL, blood or dialysate) were injected to a 2-mL glass container having a lid. Headspace gases were analyzed after ultrasonic vibration.

We chose the redox state of HSA as a marker of OS. Human serum albumin is a protein composed of 585 amino acids with a cysteine residue at position 34 containing a mercapto group (thiol/SH group) just like GSH. The mercapto group reduces other substances according to the degree of proximate OS and is oxidized itself. With respect to cysteine 34, HSA is a mixture of human mercaptoalbumin (HMA, with a non-oxidized mercapto group), human non-mercaptoalbumin-1 (HNA-1, with reversible oxidization of disulfide bonds), and human non-mercaptoalbumin-2 (HNA-2, which is strongly oxidized to the sulfinic [$–SO_2H$] or sulfonic [$–SO_3H$] forms).

The redox state of albumin was determined using high-performance liquid chromatography (HPLC) as previously reported.[5] The HPLC system consisted of an AS-8010 autosampler (Tosoh, Tokyo, Japan; 2 μL injection volume) and a CCPM double-plunger pump (Tosoh) in conjunction with a CO-8011 system controller (Tosoh). Chromatographs were obtained using a UV6000LP photodiode alley detector (detection area, 200–600 nm with 1 nm step; Thermo Electron, Waltham, MA, USA). A Shodex-Asahipak ES-502N 7C column (10 × 0.76 cm I.D., DEAE-form for ion-exchange HPLC; Showa Denko, Tokyo, Japan; column temperature, 35 ± 0.5°C) was used in this study. Elution of serum, in 0.05 M sodium acetate and

Table 3 The change of dialysate and blood hydrogen (H_2) level (p.p.b.) during hemodialysis (HD) session

		S-HD		H_2-HD	
		Dialysate	Blood	Dialysate	Blood
Pre	Inlet	0.37 ± 0.10	0.32 ± 0.03	62.89 ± 9.60	4.71 ± 20.92
	Outlet	0.38 ± 0.13	0.34 ± 0.04	47.92 ± 35.63	71.55 ± 9.03
Post	Inlet	0.35 ± 0.06	0.44 ± 0.08	54.11 ± 5.74	1.30 ± 3.48
	Outlet	0.36 ± 0.10	0.33 ± 0.08	48.25 ± 34.16	67.77 ± 7.39

H_2-HD = hemodialysis using hydrogen-enriched dialysate; S-HD = hemodialysis using standard dialysate.

0.40 M sodium sulfate (pH 4.85), was performed at a flow rate of 1.0 mL/min using a linear gradient with ethanol concentrations as follows: 0–1 minute, 0%; 1–50 minutes, 0→10%; 50–55 minutes, 10→0%; 55–60 minutes, 0%. De-aeration of the buffer solution was performed by helium bubbling.

Curve fitting of the HPLC profiles was performed using PeakFit version 4.05 simulation software (SPSS Science, Chicago, IL, USA), and each peak shape was approximated by a Gaussian function. The obtained values were used to calculate the proportions of HMA, HNA-1, and HNA-2 in total HSA (*f*[HMA], *f*[HNA-1], and *f*[HNA-2], respectively).

Statistical analysis

Values were expressed as mean ± standard deviation unless otherwise stated. StatView statistical software (version 5.0, SAS Institute, Cary, NC, USA) was used for statistical analysis. Significance of collected data was evaluated using the paired *t*-test. Differences were considered significant for values of $P < 0.05$.

RESULTS

Table 2 shows the values of dialysate pH, pCO_2 and pO_2. There were no significant differences in these values between S-HD and H_2-HD.

In the S-HD session, dialysate and blood H_2 levels were consistently <1 p.p.b. (Table 3). During the H_2-HD session, dialysate H_2 levels were maintained at ≈50 p.p.b., while blood H_2 levels at the outlet were greater than that at the inlet. This suggests that dialysate H_2 is effectively transferred to blood through the dialysis membrane.

Comparisons of the inlet and outlet of the dialyzer revealed that there were significant decreases in tGSH and rGSH, as well as significant increases in H_2O_2, during both HD sessions. Comparing inlet-blood between pre-HD and post-HD treatment revealed significant decreases in tGSH and rGSH levels in both sessions (Table 4).

Figure 2 shows the differences (Δ) in tGSH, rGSH, and H_2O_2 between the inlet and outlet of the dialyzer. No significant differences were found between S-HD and H_2-HD.

Table 5 shows the redox fraction of serum albumin in pre-inlet, pre-outlet, post-inlet, and post-outlet samples. In both HD sessions, *f*(HMA) was significantly elevated post-HD, whereas *f*(HNA-1) and *f*(HNA-2) declined significantly post-HD. In comparing the inlet and outlet of the dialyzer, it was found that the *f*(HNA-1) of both pre-outlet and post-outlet serum was significantly lower than in the inlet serum of the H_2-HD session. However, this trend was not observed in the S-HD session, suggesting that "intra-dialyzer" OS is reduced during H_2-HD.

Figure 3 shows the differences (Δ) in *f*(HMA) and *f*(HNA-1) between the inlet and outlet of the dialyzer.

Table 4 Reduced glutathione (rGSH; μg/mL), total glutathione (tGSH; μg/mL), and hydrogen peroxide (H_2O_2; nM) of each serum

		S-HD			H_2-HD		
		rGSH	tGSH	H_2O_2	rGSH	tGSH	H_2O_2
Pre	Inlet	2.1 ± 0.2	2.3 ± 0.2	8.7 ± 0.9	1.8 ± 0.2	2.0 ± 0.2	8.9 ± 1.2
	Outlet	1.1 ± 0.2*	1.2 ± 0.2*	12.9 ± 1.5*	1.1 ± 0.2*	1.1 ± 0.2*	12.7 ± 1.1*
Post	Inlet	1.7 ± 0.2	1.9 ± 0.2	9.1 ± 0.5	1.8 ± 0.2	1.9 ± 0.2	10.9 ± 0.9
	Outlet	1.2 ± 0.1**	1.3 ± 0.2**	12.9 ± 1.3**	1.2 ± 0.1**	1.3 ± 0.2**	12.4 ± 1.0**

H_2-HD = hemodialysis using hydrogen-enriched dialysate; S-HD = hemodialysis using standard dialysate.
*$P < 0.05$ vs. pre-inlet, **$P < 0.05$ vs. post-inlet.

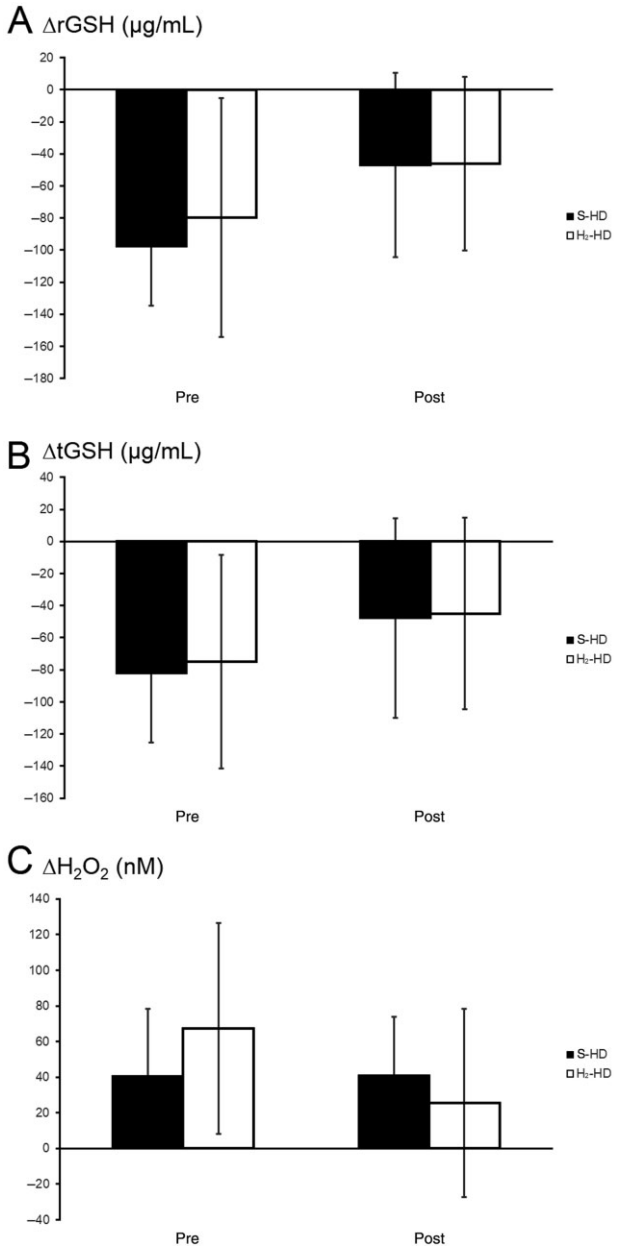

Figure 2 The effect of dialysis on the levels of (A) reduced glutathione (rGSH), (B) total glutathione (tGSH) and (C) hydrogen peroxide (H_2O_2). Differences in each parameter between the inlet and outlet (outlet—inlet) are defined as Δ. No significant differences were found between hemodialysis using standard dialysate (S-HD) and hydrogen-enriched dialysate (H_2-HD).

The post-HD Δ*f*(HMA) value was significantly higher in H_2-HD than in S-HD (1.44% ± 1.74% and −1.06% ± 1.45%, respectively). Likewise, the post-HD Δ*f*(HNA-1) value was significantly lower in H_2-HD than in S-HD (−1.55% ± 1.39% and 0.87% ± 1.26%, respectively).

DISCUSSION

The present study was conducted to examine changes in levels of rGSH, tGSH and H_2O_2, and the redox status of serum albumin after a single pass of an HD dialyzer, as well as to identify differences between two experimental groups: HD using conventional and H_2-enriched solutions. There were significant decreases in tGSH and rGSH levels, as well as a significant increase in H_2O_2 level, following a single HD session in both groups. However, we observed significant increases in the proportion of reduced albumin as we already reported,[4,5] and reciprocal decreases in the oxidative form of albumin in the H_2-HD group, whereas no reciprocal changes were found in the conventional solution group.

In this study, we observed decreased plasma GSH following HD (both S-HD and H_2-HD) session. GSH, which is a major intracellular antioxidant, participates in extracellular antioxidative property in cooperation with other thiols such as HSA and free cysteine.[23] It is proposed that HD may reduce the antioxidative capacity of plasma through loss of antioxidants such as uric acid and ascorbic acid.[15] Taken together, removal of GSH might be involved with the loss of antioxidative property during HD session.

Plasma albumin is known to play a major role as an antioxidant[24] and accumulating data show that its redox state is a good surrogate marker for the clinical prognosis of HD patients, with better survival found in patients with higher ratios of reduced albumin.[3,4] In this regard, HD-induced changes in the albumin redox state may be clinically significant. In the comparison between inlet and outlet of dialyzer, no changes were found in the albumin redox state in S-HD, whereas there was a significant increase in the proportion of reduced albumin in H_2-HD. This indicates that the elevated OS associated with the dialyzer can be partly ameliorated by H_2 loading, thereby enhancing the antioxidant capacity of plasma.

The precise mechanism responsible for H_2 loading-induced increases in the proportion of reduced albumin (increased –SH residues) remains unclear. We propose two possibilities, one being the chemical reaction of H_2 with disulfide residues. H_2 may act as a proton donor, as evinced by its capability to react with hydroxyl radicals to form H_2O. H, which is generated during the chemical reaction between H_2 and hydroxyl radicals, may be recruited to convert disulfide residues to –SH. In fact, it has been reported that H_2-enriched water, created using a water electrolysis technique, has a unique chemical

Table 5 The albumin redox of each serum

		S-HD			H_2-HD		
		f (HMA) %	*f* (HNA-1) %	*f* (HNA-2) %	*f* (HMA) %	*f* (HNA-1) %	*f* (HNA-2) %
Pre	Inlet	62.2 ± 14.6	34.2 ± 14.0	3.6 ± 0.9	60.5 ± 13.3	35.6 ± 12.5	3.9 ± 1.0
	Outlet	62.9 ± 13.2	33.4 ± 13.0	3.7 ± 0.9	63.3 ± 13.8*	32.8 ± 13.0*	3.9 ± 0.8
Post	Inlet	77.1 ± 9.5	20.0 ± 9.0	2.9 ± 0.7	74.5 ± 10.8	22.7 ± 10.5	2.8 ± 0.8
	Outlet	76.1 ± 9.6	20.9 ± 9.5	3.1 ± 0.8	75.9 ± 11.0	21.2 ± 11.0**	2.9 ± 0.6

H_2-HD = hemodialysis using hydrogen-enriched dialysate; HMA = human mercaptoalbumin; HNA-1 = human non-mercaptoalbumin-1; HNA-2 = human non-mercaptoalbumin-2; S-HD = hemodialysis using standard dialysate.
*P < 0.05 vs. pre-inlet, **P < 0.05 vs. post-inlet.

property, exhibiting superoxide dismutase and catalase-like activities,[25] and provides protection against DNA breakage in a mixture of Cu(II) and ascorbic acid *in vitro*.[25] The other possible mechanism involves the following biological process. Within the dialyzer, there is monocyte activation and excess neutrophil bursting (as evidenced by the elevation of H_2O_2 level), which drives the plasma redox state toward oxidation. The amelioration of this pathological process by H_2 loading may result in a more reductive environment. The mechanisms and clinical relevance of H_2-HD need to be addressed in future studies.

An HD system employing a water electrolysis technology was originally reported in 2004.[26] Using water electrolysis methodology, we constructed a system capable of

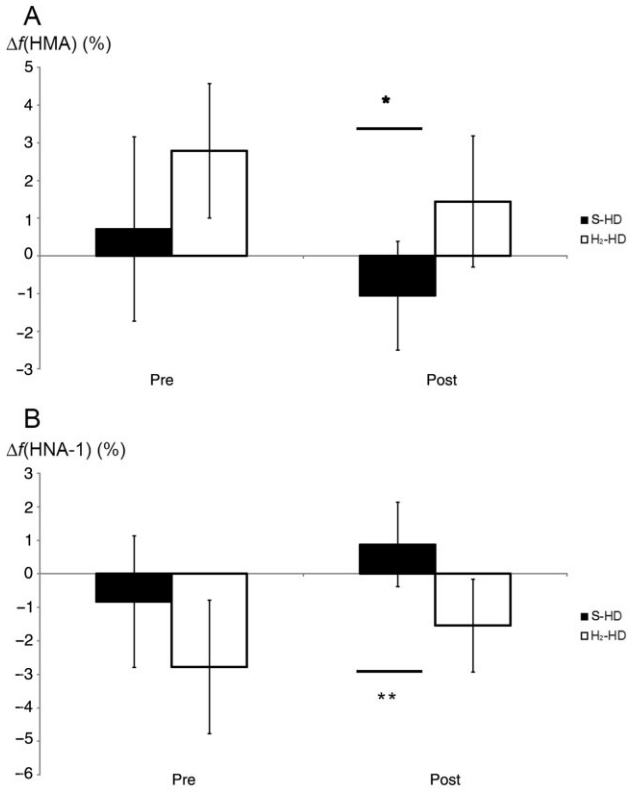

Figure 3 The effect of dialysis on the levels of human mercaptoalbumin (HMA) and human non-mercaptoalbumin-1 (HNA-1). The differences between inlet and outlet (outlet—inlet) levels are defined as (A) Δ*f*(HMA) and (B) Δ*f*(HNA-1). The post-hemodialysis (HD) Δ*f*(HMA) value was significantly higher in HD using hydrogen-enriched dialysate (H_2-HD) than in HD using standard dialysate (S-HD). Similarly, the post-HD Δ*f*(HNA-1) levels were significantly lower in H_2-HD than in S-HD. *P = 0.0025, **P = 0.0009.

constant H_2 delivering, which has been developed into a novel HD system that uses H_2-enriched electrolyzed water.[18–20] The size of an H_2 bubble is typically <1 μm in diameter, with H_2 concentrations of ≈40–100 p.p.b. At present, the system is employed in 12 dialysis facilities in Japan, and >250 patients have been treated with this system. A collaborative prospective clinical study is in progress to examine the clinical outcomes of this system as compared with the conventional system (UMIN 000004857). We think that the result of the present study strengthens the justification for using the H_2-HD system.

In conclusion, the use of H_2-enriched dialysate reduces "intra-dialyzer" OS in the clinical setting. Such suppression of OS induced by an HD session might result in the reduced incidence of CVD. Therefore, a longitudinal study to confirm the clinically beneficial effects of H_2-HD, especially in the suppression of CVD, is warranted.

Manuscript received August 2013; revised October 2013.

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