View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Kidney International, Vol. 60 (2001), pp. 2351–2359

Glyoxalase I deficiency is associated with an unusual level of advanced glycation end products in a hemodialysis patient

TOSHIO MIYATA, CHARLES VAN YPERSELE DE STRIHOU, TOSHIYUKI IMASAWA, ATSUSHI YOSHINO, YASUHIKO UEDA, HIROYUKI OGURA, KATSUYA KOMINAMI, HIROSHI ONOGI, REIKO INAGI, MASAOMI NANGAKU, and KIYOSHI KUROKAWA

Molecular and Cellular Nephrology, Institute of Medical Sciences and Department of Medicine, Tokai University School of Medicine, and Bohsei-Hiratsuka Clinic, Kanagawa, and Division of Nephrology and Endocrinology, University of Tokyo School of Medicine, Tokyo, Japan; and Service de Nephrologie, Université Catholique de Louvain, Brussels, Belgium

Glyoxalase I deficiency is associated with an unusual level of advanced glycation end products in a hemodialysis patient.

Background. Advanced glycation of proteins and their attendant advanced glycation end products (AGEs) contribute to the complications associated with diabetes mellitus or uremia. Regulatory mechanisms of AGE formation in vivo remain an issue of particular interest. We investigated a role of the glyoxalase detoxification system of precursor reactive carbonyl compounds (RCOs) in the in vivo AGE formation.

Methods. Plasma levels of AGEs [pentosidine and N^{ε}-carboxymethyllysine (CML)], their RCO precursors, D-lactate (the final product resulting from the glyoxalase detoxification pathway), as well as of various compounds known to generate AGE precursors and surrogate markers for oxidative stress (antioxidant enzymes and glutathione), were measured in both hemodialysis (HD) patients and normal subjects. The activity and protein expression of glyoxalase I, an enzyme essential for the detoxification of α-oxoaldehydes, in red blood cells (RBC) were also examined.

Results. In one 69-year-old lady who had been on hemodialysis (HD) for three years and had suffered from recurrent cardiovascular complications despite the absence of significant risk factors, plasma levels of pentosidine (77.3 ± 2.4 pmol/mg protein) and CML (330.8 ± 8.2 pmol/mg protein) were markedly elevated as compared to other HD patients ($N = 20$: 26.6 ± 11.8 pmol/mg protein for pentosidine and 224.4 ± 51.7 pmol/mg protein for CML). The plasma level of RCO precursors for pentosidine and CML was also higher in this patient than in other HD patients. Further investigation disclosed a very low activity in RBC of glyoxalase I (1.5 ± 0.4 mU/10^6 RBC), as compared to other HD patients (3.9 ± 0.6 mU/10^6 RBC) or normal subjects (4.0 ± 0.6 mU/10^6 RBC). The glyoxalase I protein level, assessed in RBC by immunoblot analysis with a specific antibody, was markedly lower than that observed in HD patients and normal subjects. The causes of this deficiency remain unknown. Nucleotide sequencing of the products of reverse transcription-polymerase chain reaction from the patient's mononuclear cells revealed no genetic mutation within the coding region of the glyoxalase I gene. Plasma D-lactate level was also in the lower range (0.18 ± 0.03 mg/dL) of the values measured in the other HD patients (0.27 ± 0.09 mg/dL) and normal subjects (0.35 ± 0.12 mg/dL). The plasma levels of various compounds known to generate AGE precursors (glucose, lipids and ascorbic acid) were either normal or low. The surrogate markers for oxidative stress such as antioxidant enzymes (glutathione peroxidases and superoxide dismutase) and glutathione were all within the range observed in the other HD patients.

Conclusion. The unusually high levels of AGEs in this patient implicate a deficient glyoxalase detoxification of RCO precursors. The present clinical observation implicates, to our knowledge for the first time, the glyoxalase detoxification system and, in particular, glyoxalase in the actual level of AGEs in a uremic patient.

Key words: carbonyl compound, carbonyl stress, diabetes mellitus, uremia, reactive carbonyl compounds, glyoxalase pathway.

Received for publication April 16, 2001
and in revised form June 28, 2001
Accepted for publication July 5, 2001

© 2001 by the International Society of Nephrology

Advanced glycation end products (AGEs) contribute to the development of complications associated with diabetes and uremia [1–4]. In uremic patients, their level is dictated by the accumulation of precursor reactive carbonyl compounds (RCOs) [5], the so-called "uremic carbonyl stress" [6, 7].

Several mechanisms might account for RCO accumulation in renal failure. Production of RCOs might be enhanced by the oxidative stress reported in uremic patients [8]. This hypothesis is supported by the positive correlations demonstrated in uremic serum between pentosidine, an AGE compound, and markers of oxidative stress such as dehydroascorbate [9], advanced oxidation protein products (AOPP) [10] and superoxide dismutase (SOD) [11], as well as by the negative correlation reported between serum pentosidine and glutathione peroxidase levels [11]. The oxidative stress hypothesis, however, does not account for the raised levels of two RCOs, 3-deoxyglucosone [12] and methylglyoxal [13],

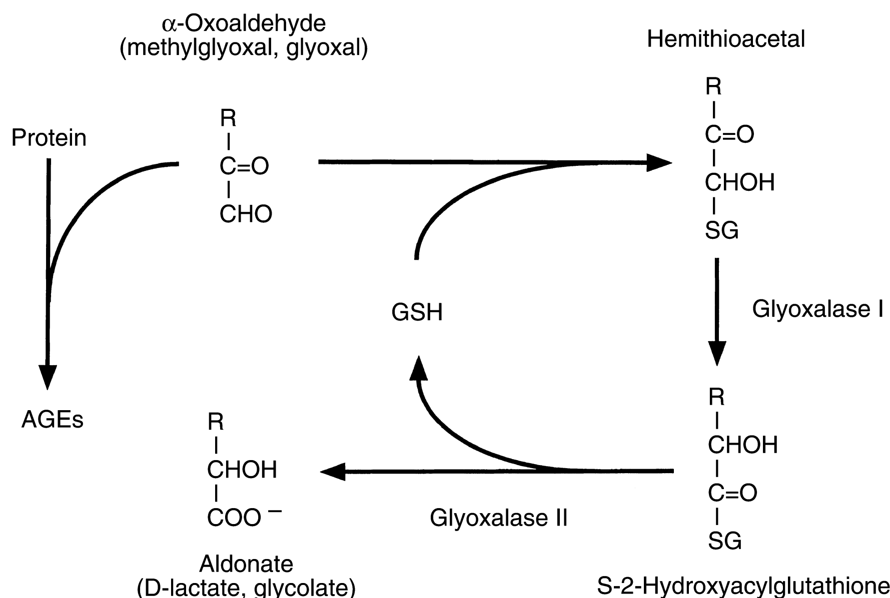

Fig. 1. The glyoxalase detoxification pathway. α-Oxoaldehydes, such as methylglyoxal (glyoxal), react reversibly with the thiol group of glutathione to be subsequently metabolized by glyoxalases I and II into glutathione and D-lactate (glycolate). Decrease in glutathione levels or in glyoxalase activity might therefore raise the level of a wide range of RCOs with the attendant formation of AGEs.

both of which are derived from non-oxidative chemistry and react with proteins to form AGEs.

Alternatively, RCOs might arise in uremia as a consequence of an impaired detoxification. RCOs are metabolized by several enzymatic pathways, such as aldose reductase, aldehyde dehydrogenases, and the glyoxalase pathway [14]. The latter pathway is believed to be of particular importance. It catalyzes the detoxification of α-oxoaldehydes (RCOCHO), such as glyoxal, methylglyoxal and 3-deoxyglucosone, to corresponding aldonic acids [RCH(OH)COOH] (Fig. 1). To date, however, there is no evidence that the RCO detoxification mechanism might influence in vivo RCO and therefore serum AGE levels. The present clinical observation implicates, to our knowledge for the first time, the glyoxalase detoxification system and in particular glyoxalase, an essential enzymatic element in the glyoxalase pathway, in the actual level of AGEs in a uremic patient.

Case report

A 69-year-old Japanese woman had been on regular hemodialysis (HD) for three years. In 1985, at the age of 55 years, she was told that her renal function was decreased, probably due to chronic glomerulonephritis (no renal biopsy). Blood pressure, and serum glucose and lipid levels were normal. No drugs were given. Twelve years later, in July 1997, atherosclerosis obliterans of the common iliac artery was discovered on angiography and treated by a percutaneous transluminal angioplasty. Renal function decreased progressively thereafter and HD was started in May 1998. During the subsequent three years, the patient suffered from several cardiovascular complications, including arteriovenous (AV) fistula troubles, cerebral infarction in July 1999, and unstable angina. In January 2000, a coronary angiography showed a 90% stenosis of the left anterior descending coronary artery. As cardiovascular risk factor, she had smoked 5 cigarettes a day for 10 years, but stopped 10 years earlier. She had no family history of diabetes mellitus. Hypertension developed after the onset of HD, but was easily controlled by nifedipine and doxazosin mesilate. Body mass index was 16.4 (height 149 cm, weight 36.5 kg). Biochemical data in December 1999 included fasting plasma glucose (102 mg/dL), serum total cholesterol (218 mg/dL), low-density-lipoprotein (129 mg/mL), triglyceride (189 mg/dL), high-density-lipoprotein (67 mg/dL), lipoprotein (18.7 mg/dL), uric acid (7.1 mg/dL), ascorbic acid (<1.5 μg/mL), and C-reactive protein (0.4 mg/dL). The red blood cell (RBC) count was $2.06 \times 10^6/\mu L$ [hemoglobulin (Hb) 7.2 g/dL, hematocrit (Hct) 22.3%] and hemoglobulin A1C (HbA_{1c}) was 5.4%. The discovery of strikingly elevated plasma levels of pentosidine initiated further investigations described hereafter.

METHODS

Plasma samples

Fresh heparinized plasma samples were obtained from the index case, from 4 subjects (3 male) with a mean age of 35.0 ± 9.6 years and normal renal function, and from 20 non-diabetic HD patients (11 male) with a mean age of 55.2 ± 16.3 years. Informed consent was obtained from all patients. Normal renal function was defined as a serum creatinine level below 1.0 mg/dL (88.4 μmol/L) and the absence of proteinuria by a negative Albustix (Bayer-Sankyo Co. Ltd., Tokyo, Japan). HD was per-

formed in all investigated uremic patients with a modified-cellulose membrane (surface area 1.0 to 2.0 m^2) and a dialysate containing 5.56 mmol/L glucose, 30 mmol/L bicarbonate and 8 mmol/L acetate. Protein concentration was measured by a Bio-Rad protein assay (Richmond, CA, USA) with human albumin as a standard.

In vitro incubation experiment

Plasma samples were ultrafiltrated through a filter with a 5000 D cut-off value (Ultrafree CL-LCC; Nihon Millipore Ltd., Tokyo, Japan). Plasma ultrafiltrates (1.0 mL) were sterilized with a 0.22-μm pore filter, fortified with essentially fatty acid-free grade bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, MO, USA) to the final concentration of 30 mg/mL, and subsequently incubated under air in sealed sterile 1.5 mL plastic tubes at 37°C for two and one weeks, followed by the determination of pentosidine and CML contents, respectively. In some experiments, incubation was performed in the presence of 10 mmol/L of either aminoguanidine (Tokyo Chemical Industry, Tokyo, Japan) [15] or (±)-2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide (OPB-9195; developed by Fujii Memorial Research Institute, Otsuka Pharmaceutical, Ohtsu, Japan) [16].

Pentosidine and CML

Pentosidine content was determined by high-performance liquid chromatographic (HPLC) assay as described previously [17]. In brief, the sample was lyophilized, hydrolyzed in 100 μL of 6 N HCl for 16 hours at 110°C under nitrogen, followed by neutralization with 100 μL of 5 N NaOH and 200 μL of 0.5 mol/L sodium phosphate buffer (pH 7.4), filtered through a 0.5 μm filter, and diluted with phosphate-buffered saline (PBS). A sample (corresponding to 25 μg of proteins) was injected into an HPLC system and fractionated on a C18 reverse-phase column. The effluent was monitored at an excitation-emission wavelength of 335/385 nm using a fluorescence detector (RF-10A: Shimadzu). Synthetic pentosidine was used to obtain a standard curve. Limits of detection were 2 pmol of pentosidine per mg of protein.

For quantitation of CML content, samples (100 μL) were diluted with an equal volume of 0.2 mol/L sodium borate (pH 9.1), followed by addition of 20 μL 1 mol/L $NaBH_4$ in 0.1 N NaOH. Reduction was carried out for four hours at room temperature, and proteins were precipitated by addition of an equal volume of 20% trichloroacetic acid and pelleted by centrifugation at 2,000 × *g* for five minutes. The pellet was washed with 500 μL 10% trichloroacetic acid. Heavy labeled internal standards (d_4-CML) were added and the samples hydrolyzed in 0.3 mL 6 N HCl at 110°C for 16 hours. The hydrolysates were dried under a stream of nitrogen. CML content in the hydrolysates was determined as its *N,O*-trifluoroacetyl methyl esters by selected-ion monitoring gas chromatography/mass spectrometry (GC/MS), as described previously [18]. The CML and d4-CML standards [19] were gifts from Dr. John W. Baynes. The limit of detection was 1.0 pmol of CML per mg of protein.

Antioxidant enzymes and glutathione

The levels of Cu/Zn-superoxide dismutase and glutathione peroxidase were measured in the plasma using kits (Cu/Zn-superoxide dismutase; Amersham Pharmacia Biotech, Little Chalfont, UK; glutathione peroxidase; Oxis International, Portland, OR, USA).

The concentration of glutathione in RBC was measured by a kit (BIOXYTECH GSH-400; Oxis International). This assay involves formation of thioethers between 4-chloro-1-methyl-7-trifluoromethyl-quinolinium methylsulfate and glutathione, and transformation of the thioether into a chromophoric thione, which has a maximal absorbance wavelength at 400 nm.

Glyoxalase I activity

Red blood cells were obtained by centrifugation from heparinized blood samples, washed three times with saline, and suspended in saline (10% hematocrit stock suspension).

The activity of glyoxalase I in RBC was assayed according to the method by McLellan et al [20]. Briefly, the red blood cell suspension (10% hematocrit) was lysed with 4 volumes of ice-cold distilled water. The membrane fragments were sedimented by centrifugation and lysates were assayed for glyoxalase I activity. Glyoxalase I activities are given in units/10^6 RBC, where 1 unit is the amount of enzyme required to catalyze the formation of 1 μmol *S*-D-lactoylglutathione per minute.

Glyoxalase I protein level: Immunoblotting

The lysates from 2×10^6 RBC were separated by SDS-PAGE using a 4 to 20% acrylamide gel. Proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad Laboratories, Hercules, CA, USA) electrophoretically. The membrane was blocked overnight at 4°C with PBS containing 0.05% Tween and 2% BSA, incubated with rabbit anti-human glyoxalase I IgG (1.0 μg/mL) [21], (kindly provided by Dr. Sulabna Ranganathan, Department of Pharmacology, Fox Chase Cancer Center, PA, USA) for one hour at room temperature, and washed with PBS containing 0.05% Tween 20. The membrane was then incubated with 1:5,000 diluted alkaline phosphate conjugated goat anti-rabbit IgG (Cappel Laboratory, Durham, NC, USA) for one hour at room temperature, followed by washing and development with *p*-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate solution (Bio-Rad Laboratories).

D-Lactate

D-Lactate was measured in plasma samples by the method of Brandt et al [22]. This assay is based on the

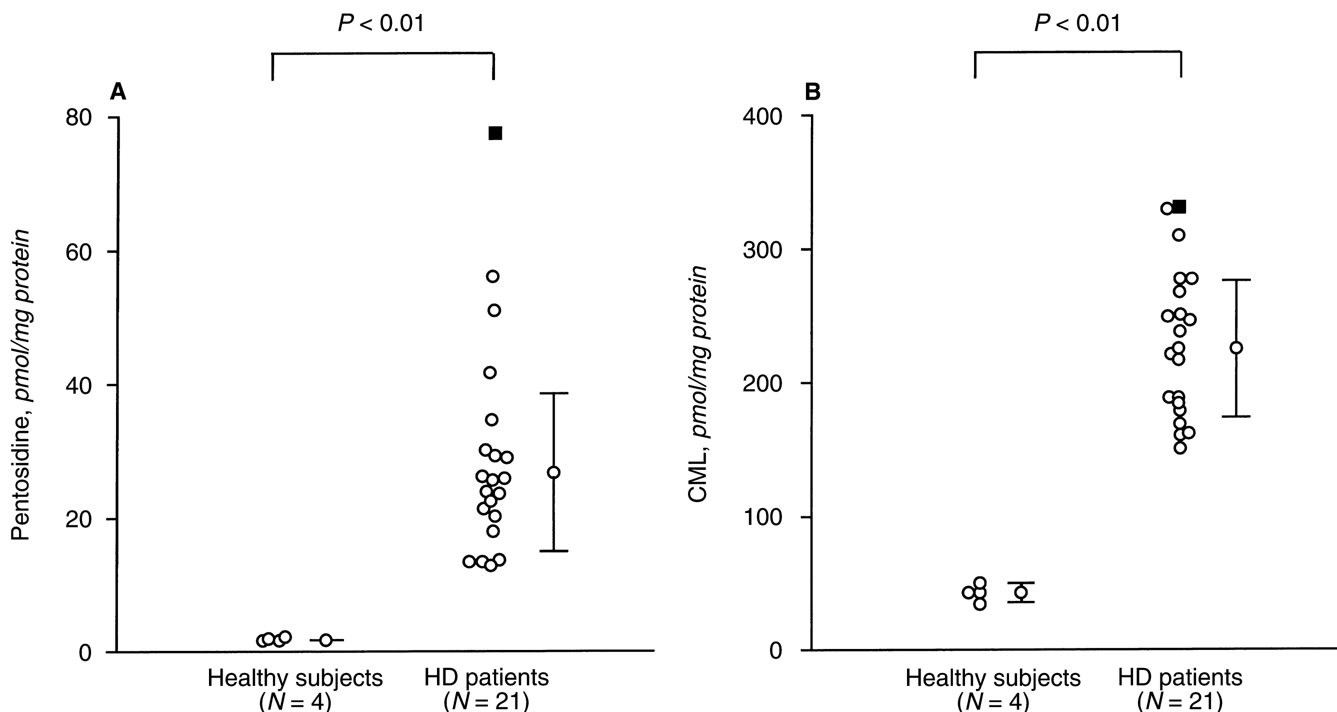

Fig. 2. Plasma pentosidine and N^{ε}-carboxymethyllysine (CML) levels in normal and hemodialysis (HD) subjects. Pentosidine (*A*) and CML (*B*) contents in acid hydrolysates of plasma from normal subjects ($N = 4$), non-diabetic HD patients ($N = 20$), and the index patient (■) were determined by HPLC or GC/MS assay, respectively. Data are expressed as means ± SD.

enzymatic oxidation of D-lactate with a specific D-lactate dehydrogenase coupled to reduction of nicotinamide adenine dinucleotide (NAD^+) with the spectrophotometric measurement of NADH at 340 nm.

Genetic analysis for glyoxalase I gene

Heparinized peripheral blood samples were collected from the index patient and two healthy volunteers. Mononuclear cells were isolated from the blood by Ficoll centrifugation. RNA isolated from mononuclear cells by a kit (ISOGEN: Nippon Gene, Tokyo, Japan) were reverse-transcribed with oligo$(dT)_{12\text{–}18}$ primers with RNase H-free reverse transcriptase (Superscript II: Gibco BRL, Gaithersburg, MD, USA). The coding region of glyoxalase I was then amplified by polymerase chain reaction (PCR) with primers, sense (5′-GTA GTG TGG GTG ACT CCT CCG TTC CTT GGG-3′) and antisense (5′-TCC TTT CTT CTG AAA TCT CAA AGG AGA ATT CTC-3′) with an initial denature at 94°C for five minutes, followed by 30 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for one minute.

The amplified products from the coding region of glyoxalase I (648 bp) were subcloned into plasmids for determination of their nucleotide sequences by ABI PRISM™ 377 DNA sequencer (Perkin Elmer Biosystems, Foster, CA, USA). Both strands of the cloned products were sequenced in 20 clones from each subject.

Statistical analysis

Data are expressed as means ± SD. The Mann-Whitney test was used for a statistical evaluation of significant difference between HD patients (not including the index patient) and normal healthy subjects. Correlation was assessed by linear regression analysis. A *P* value below 0.05 was considered statistically significant.

RESULTS

Elevated plasma levels of AGEs and their precursor RCOs

The patient's plasma pentosidine level reached 77.3 ± 2.4 pmol/mg protein, a value that far exceeded those observed in the HD (26.6 ± 11.8 pmol/mg) or normal healthy (1.76 ± 0.23 pmol/mg protein) subjects (Fig. 2A). The plasma CML level reached 330.8 ± 8.2 pmol/mg protein, a remarkably high value when compared with other HD (224.4 ± 51.7 pmol/mg protein) and normal (43.0 ± 6.5 pmol/mg protein) subjects (Fig. 2B). The index patient's elevated levels were documented on three different occasions.

To assess the levels of precursor RCOs accumulated in the circulation, we incubated ultrafiltrated plasma fortified with BSA for two and one weeks, and measured the in vitro generation of pentosidine and CML, respectively. Generation of both pentosidine and CML was

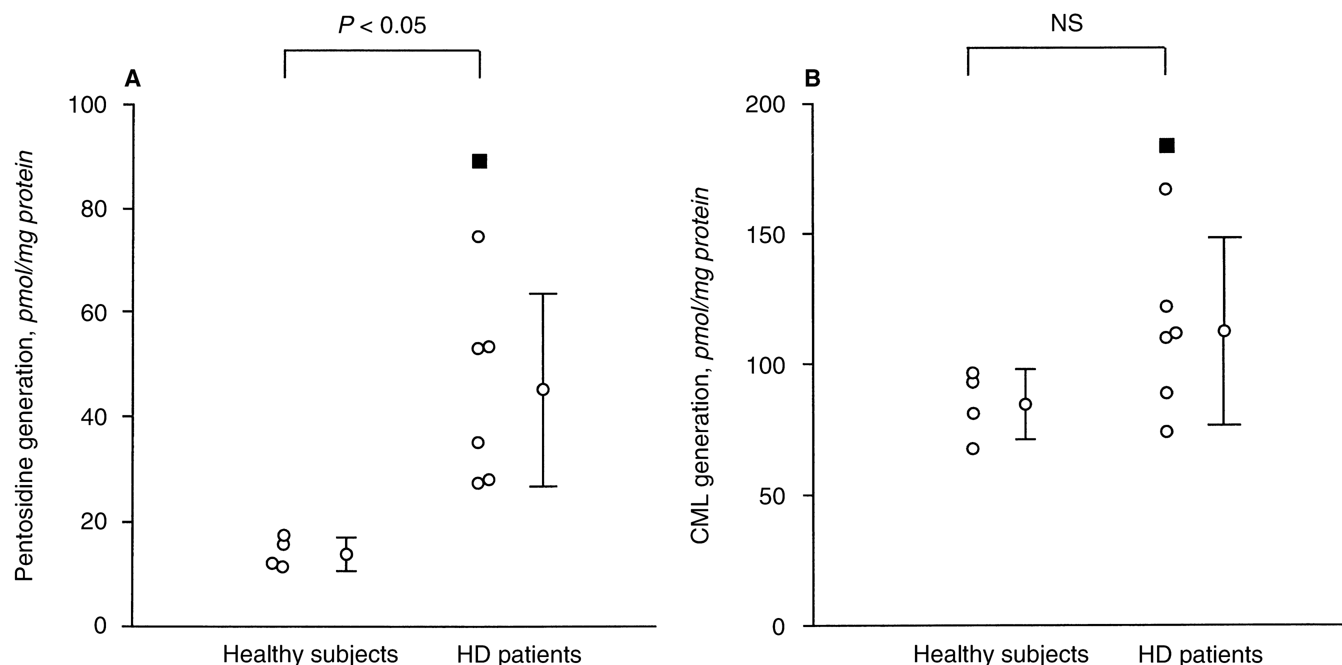

Fig. 3. Generation of pentosidine and CML upon incubation of plasma ultrafiltrates. Plasma ultrafiltrates (<5000 D), obtained from normal subjects ($N = 4$), non-diabetic HD patients ($N = 6$) and the index patient (■), were fortified with BSA (final concentration: 30 mg/mL) and incubated at 37°C for one (CML) or two (pentosidine) weeks under air. Pentosidine (*A*) and CML (*B*) contents in acid hydrolysates were determined by HPLC or GC/MS assay, respectively. Data are expressed as means ± SD. NS, not significant.

much higher in this patient than in other HD patients or normal subjects (Fig. 3). The addition of aminoguanidine or OPB-9195, both of which inhibit the carbonyl amine reactions [16], inhibited pentosidine and CML generation during the incubation of plasma ultrafiltrates (data not shown). These results demonstrate that the plasma precursors of pentosidine and CML are indeed RCOs and that their levels are remarkably higher in the index patient than in other HD patients and normal subjects.

Causes of elevated levels of RCOs precursors of AGEs

To understand why AGEs and AGE precursor RCO levels were so high in this patient, further biochemical analyses were performed. As pointed out in the case report, the levels of various compounds known to generate AGE precursors such as glucose, triglycerides, and ascorbic acid were either within normal limits or low.

Oxidative stress

The plasma activities of glutathione peroxidase and superoxide dismutases, two antioxidant enzymes, were used as an index of associated oxidative stress. Their levels in the index patient (plasma glutathione peroxidase 14.9 μg/mL; plasma Cu, Zn-SOD, 79.6 ng/mL; erythrocyte Cu, Zn-SOD, 6.10 ng/10^6 RBC) were similar to those measured in other HD patients (plasma glutathione peroxidase 14.8 ± 2.97 μg/mL; plasma Cu, Zn-SOD, 71.5 ± 25.7 ng/mL; erythrocyte Cu, Zn-SOD, 6.04 ± 0.65 ng/10^6 RBC).

Glyoxalase pathway for the detoxification of RCO precursors

The RBC level of glutathione, an essential element in the glyoxalase pathway, reached 0.131 ± 0.012 nmol/10^6 RBC in the index patient, a value well within the range observed in other HD patients (0.145 ± 0.024 nmol/10^6 RBC; Fig. 4A), as well as in normal subjects (0.141 ± 0.015 nmol/10^6 RBC). Glutathione levels expressed per blood volume were similar in the index patient (0.30 ± 0.03 μmol/mL) and in HD patients (0.45 ± 0.11 μmol/mL) but significantly lower than in normal subjects (0.68 ± 0.07 μmol/mL) as a result of the concomitant anemia.

The activity of the glyoxalase I enzyme, another key element of the glyoxalase pathway, was measured. It is of note that the glyoxalase I activity in RBC was markedly lower in the index patient (1.5 ± 0.4 mU/10^6 RBC) than in the other HD patients (3.9 ± 0.6 mU/10^6 RBC) or normal subjects (4.0 ± 0.6 mU/10^6 RBC; Fig. 4B). Similar results were obtained on three different days in the index patient.

The protein level of glyoxalase I in RBC was determined by immunoblot analysis with a specific antibody. The level in the index patient was almost half of that measured in other HD and normal subjects (Fig. 5).

In agreement with the decreased glyoxalase I activity

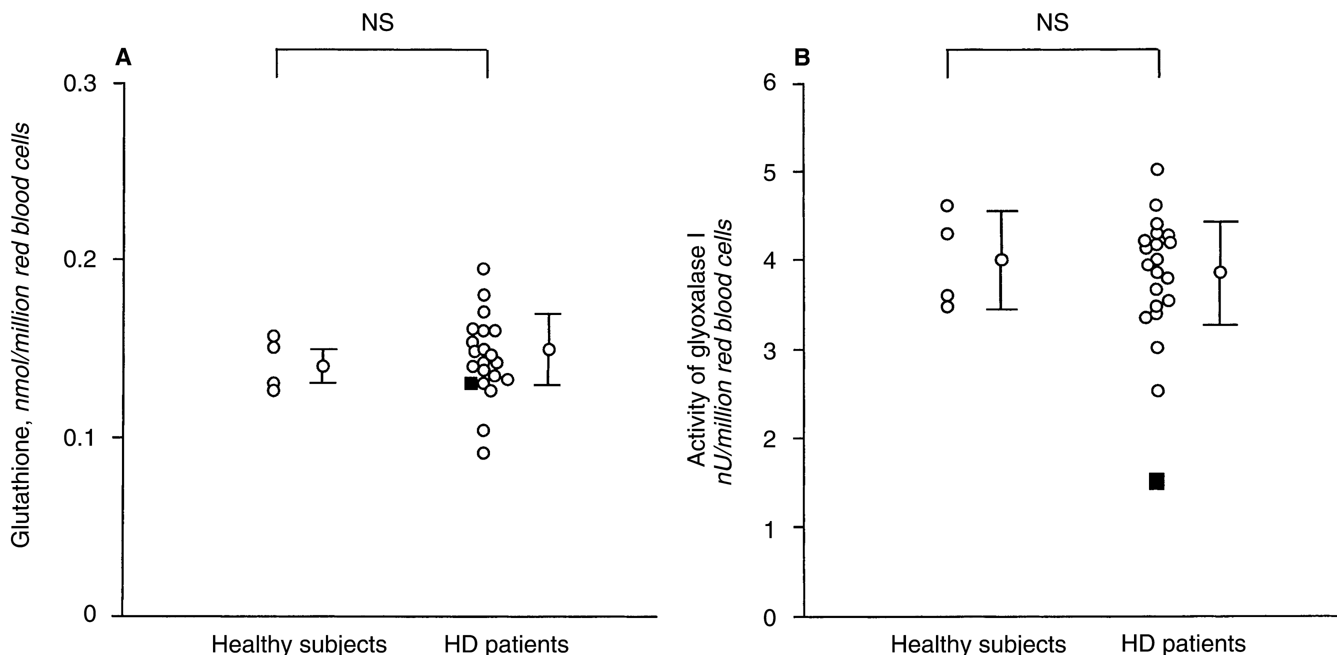

Fig. 4. Glutathione levels (*A*) and glyoxalase I activity (*B*) in RBC. (A) Glutathione concentrations in RBC from normal subjects ($N = 4$), non-diabetic HD patients ($N = 20$) and the index patient (closed square) were determined by the spectrophotometric assay. (B) The red blood cell lysates were obtained from normal subjects ($N = 4$), non-diabetic HD patients ($N = 20$) and the index patient (■), and the glyoxalase I activities were assayed. NS, not significant.

and its attendant impaired detoxification of RCOs, the plasma level of D-lactate, a final product of the glyoxalase pathway, measured in the index case (0.18 ± 0.03 mg/dL) was within the lower part of the range observed in other HD patients (0.27 ± 0.09 mg/dL) and normal subjects (0.35 ± 0.12 mg/dL; Fig. 6).

Genetic analysis

The nucleotide sequences of the coding region of glyoxalase I were determined in the index patient. All nucleotide sequences of the 20 cloned products of reverse transcription-polymerase chain reaction (RT-PCR) were completely identical to those reported for glyoxalase I. No genetic mutation within the coding region of the glyoxalase I gene was thus identified in the index case.

DISCUSSION

The present case report provides suggestive first evidence that AGE levels can be markedly influenced by the activity of the detoxification systems clearing the RCO precursors of AGEs.

The plasma levels of two representative AGEs, pentosidine and CML, were unusually elevated in our index patient. Interestingly, the correlation reported between these two species [18], extended to these very high levels (Fig. 7A). This observation strongly suggests the presence of an underlying common cause.

We have previously documented the accumulation of RCO precursors of AGEs in uremic serum, the so-called uremic carbonyl stress, and demonstrated that it played a critical role in determining serum AGE levels [5–7]. In the present patient, we demonstrate that the markedly elevated AGE levels are associated with a similarly raised carbonyl stress. Indeed, the AGE forming potential of the index patient's serum, taken as a marker of circulating RCOs, is higher than those of other HD patients.

It remains to be seen whether the marked rise in RCOs observed in our patient is due to an enhanced production or to a decreased removal as compared to other HD patients. Enhanced production appears unlikely in view of the normal serum levels of glucose and lipids, both precursors of RCOs. Although a recent study suggested that mitochondrial reactive oxygen species are involved in the intracellular formation of methylglyoxal-derived AGEs [23], an augmented RCO production due to an increased oxidative stress is also unlikely, as markers of oxidative stress such a glutathione peroxidase and superoxide dismutase were similar in the index case and in other HD patients.

The alternative hypothesis of an impaired detoxification is more attractive [6]. We have investigated only the glyoxalase pathway, which is believed to play an important role. Reduced glutathione contributes to the activity of this pathway. Methylglyoxal, for example, reacts reversibly with its thiol group and is subsequently metabolized by glyoxalases I and II into glutathione and

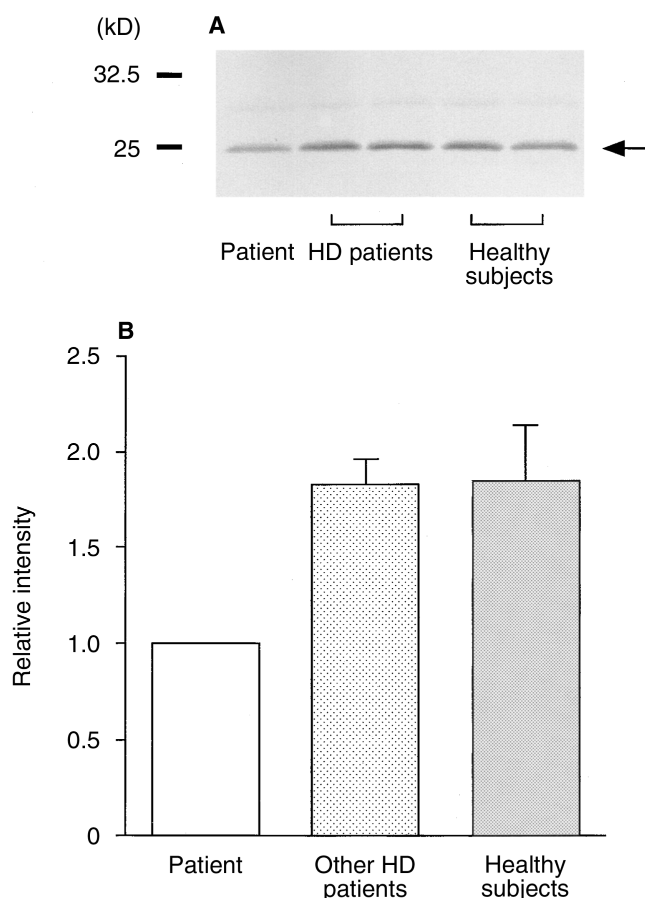

Fig. 5. Immunochemical detection of glyoxalase I in RBC. The cell lysates from normal subjects ($N = 2$), non-diabetic HD patients ($N = 2$) and the index patient were resolved by 4 to 20% SDS-PAGE, followed by immunoblotting with anti-glyoxalase I antibody (*A*). (*B*) Ratios of intensities of glyoxalase I level calculated by densitometry.

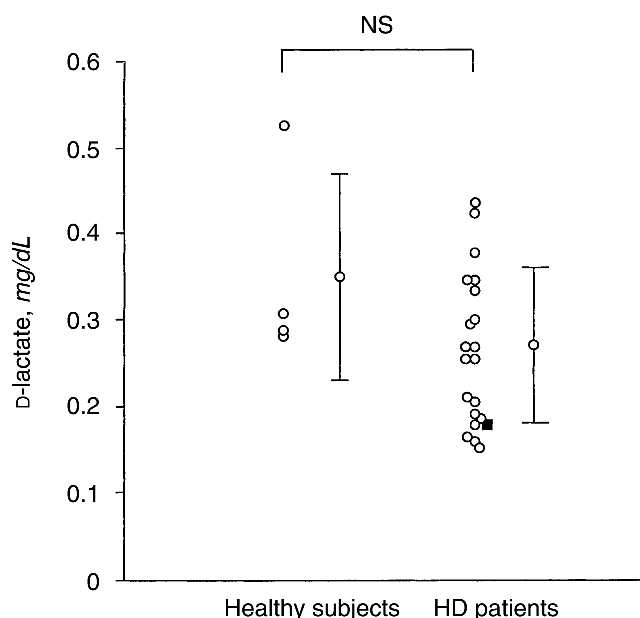

Fig. 6. Plasma D-lactate levels. D-Lactate concentrations in plasma from normal subjects ($N = 4$), non-diabetic HD patients ($N = 20$) and the index patient (■) were determined by the spectrophotometric assay. NS, not significant.

D-lactate (Fig. 1). Decreased levels of glutathione might therefore raise the serum level of a wide range of RCOs. This was not the case, since the RBC glutathione level was similar in our patient and in the other HD patients.

The most striking observation in our patient is the markedly reduced level of glyoxalase I activity. This reduction is confirmed by the immunoblot analysis of protein levels of glyoxalase I in RBC and fits with the low serum level of D-lactate. This data suggest a causal link between glyoxalase I activity and serum concentration of the two AGEs.

The causes of a reduced level of glyoxalase I in our patient remain to be determined. It might be suggested that our case is only an outlier in a normal distribution of activity. While this possibility cannot be ruled out, it does not fit with the observation that the index case is clearly outside the population of HD patients in the graphs relating glyoxalase I and either pentosidine (Fig. 7B) or CML (Fig. 7C). Alternatively, the enzyme level may be abnormally low as a consequence of a mutated gene or of another gene regulating product. Nevertheless, nucleotide sequencing of the products of RT-PCR from mononuclear cells of our patient revealed no genetic mutation within the coding region of the glyoxalase I gene. The possibility remains that some genetic mutation exists in the 5′ or 3′ region (promotor/enhancer region) of the glyoxalase I gene or in another genomic region affecting the stability or expression of glyoxalase I mRNA. At any rate, the reproducibility of the observed abnormalities in our index case clearly points to a real phenomenon.

The present data demonstrate that a lowered glyoxalase I activity is accompanied in a uremic subject by an augmented level of RCOs and of AGEs. It remains to be seen whether the glyoxalase pathway regulates AGE level in uremia. The observation that neither pentosidine nor CML levels are correlated with the level of glyoxalase I activity usually present in HD patients, that is, outside our patient, suggests that this is not the case for this enzyme. The possibility that other detoxification steps limit the clearance of RCO precursors of AGEs is yet to be explored.

Nevertheless, the evidence that unusually low levels of glyoxalase I are associated with strikingly elevated levels of AGEs and RCO precursors raises the possibility that maneuvers augmenting glyoxalase I activity might lower RCO and AGE levels and thus opens new therapeutic avenues.

Our index patient's evolution was marred by multiple cardiovascular complications despite the virtual absence of predisposing factors, except for a ten-year mild expo-

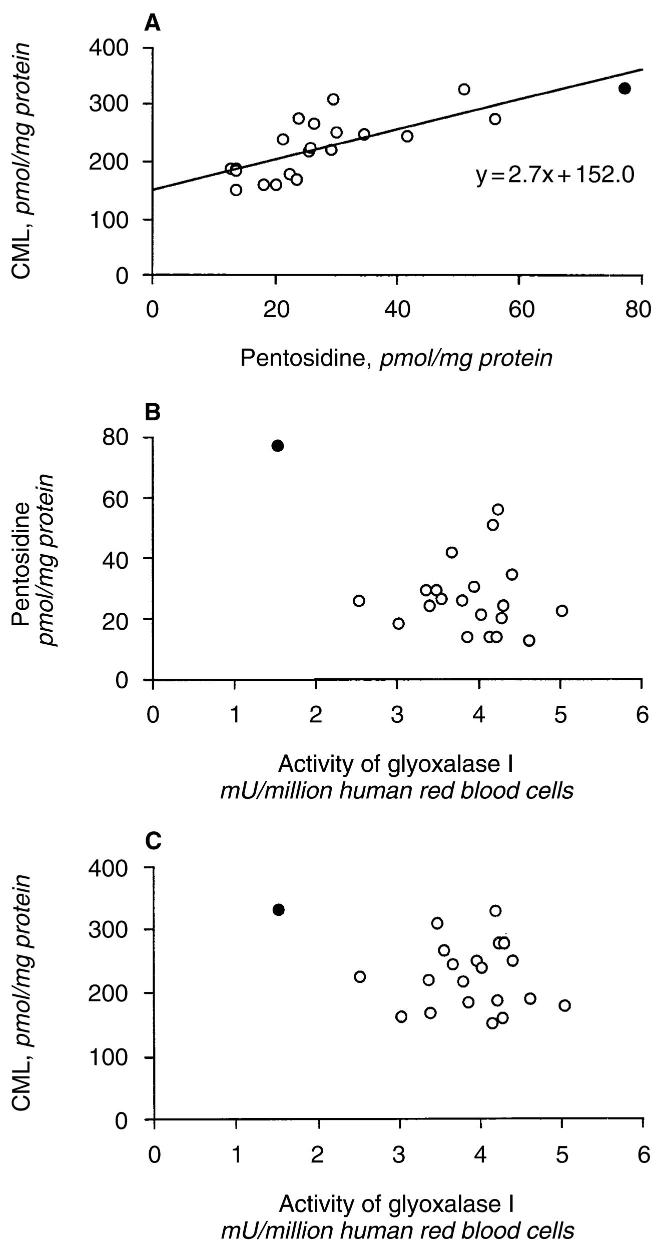

Fig. 7. Correlations in HD patients between plasma pentosidine and CML (*A*), and between plasma pentosidine and RBC glyoxalase I (*B*), and between plasma CML and RBC glyoxalase I (*C*). The symbol (●) denotes the index patient. (*A*) Plasma pentosidine levels were correlated with those of CML in HD patients ($N = 21$, $r = 0.77$, $P < 0.001$).

sure to smoking interrupted ten years earlier and a marginal rise in serum triglycerides, common in hemodialysis patients. The association of this evolution with unusually elevated AGE and RCO levels is reminiscent of several reports that advanced glycation plays a role in the development of atheromatosis. These results support the need for newer treatments aimed at lowering AGE levels not only in patients on dialysis but also in preterminal renal failure.

ACKNOWLEDGMENTS

This study was supported by grants from Research for the Future Program of the Japan Society for the Promotion of Science (96L00303) and from the Japanese Ministry of Health and Welfare for Research on Health Services (H10-079). We thank Dr. Sulabna Ranganathan for kindly providing the anti-glyoxalase I antibody used in this study, and Drs. John W. Baynes and Suzsane R. Thorpe for helpful discussion.

Reprint requests to Toshio Miyata, M.D., Ph.D., Molecular and Cellular Nephrology, Institute of Medical Sciences and Department of Medicine, Tokai University School of Medicine, Bohseidai, Isehara, Kanagawa 259-1193, Japan.
E-mail: t-miyata@is.icc.u-tokai.ac.jp

APPENDIX

Abbreviations used in this paper are: AGE, advanced glycation end product; AOPP, advanced oxidation protein products; BSA, bovine serum albumin; CML, N^{ε}-carboxymethyllysine; HD, hemodialysis; HPLC, high-pressure liquid chromatography; RBC, red blood cell; RCO, reactive carbonyl compound; RCOCHO, α-oxoaldehydes; RCH(OH)-COOH, aldonic acids; RT-PCR, reverse transcription-polymerase chain reaction; SOD, superoxide dismutase.

REFERENCES

1. Makita Z, Radoff S, Rayfield EJ, *et al*: Advanced glycosylation end products in patients with diabetic nephropathy. *N Engl J Med* 325:836–842, 1991
2. Sell DR, Lapolla A, Odetti P, *et al*: Pentosidine formation in skin correlates with severity of complication in individuals with long standing IDDM. *Diabetes* 41:1286–1292, 1992
3. McCance DR, Dyer DG, Dunn JA, *et al*: Maillard reaction products and their relation to complications in insulin-dependent diabetes mellitus. *J Clin Invest* 91:2470–2478, 1993
4. Miyata T, Jadoul M, Kurokawa K, van Ypersele de Strihou C: β2-Microglobulin in renal disease. *J Am Soc Nephrol* 9:1723–1735, 1998
5. Miyata T, Ueda Y, Yamada Y, *et al*: Carbonyl stress in uremia: Accumulation of carbonyls accelerate the formation of pentosidine, an advanced glycation end product. *J Am Soc Nephrol* 9:2349–2356, 1998
6. Miyata T, van Ypersele de Strihou C, Kurokawa K, Baynes JW: Alterations in non-enzymatic biochemistry in uremia: Origin and significance of "carbonyl stress" in long-term uremic complications. *Kidney Int* 55:389–399, 1999
7. Miyata T, Kurokawa K, van Ypersele de Strihou C: Advanced glycation and lipoxidation end products: Role of reactive carbonyl compounds generated during carbohydrate and lipid metabolism. *J Am Soc Nephrol* 11:1744–1752, 2000
8. Miyata T, Maeda K, Kurokawa K, van Ypersele de Strihou C: Oxidation conspires with glycation to generate noxious advanced glycation end products in renal failure. *Nephrol Dial Transplant* 12:255–258, 1997
9. Miyata T, Wada Y, Cai Z, *et al*: Implication of an increased oxidative stress in the formation of advanced glycation end products in patients with end-stage renal failure. *Kidney Int* 51:1170–1181, 1997
10. Witko-Sarsat V, Friedlander M, Capeillere-Blandin C, *et al*: Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int* 49:1304–1313, 1996
11. Ueda Y, Miyata T, Hashimoto T, *et al*: Implication of altered redox regulation by antioxidant enzymes in the increased plasma pentosidine, an advanced glycation end product, in uremia. *Biochem Biophys Res Commun* 245:785–790, 1998
12. Niwa T, Takeda N, Miyazaki T, *et al*: Elevated serum levels of 3-deoxyglucosone, a potent protein-cross-linking intermediate of the Maillard reaction, in uremic patients. *Nephron* 69:438–443, 1995
13. Odani H, Shinzato H, Matsumoto Y, *et al*: Increase in three α, β-dicarbonyl compound levels in human uremic plasma: Specific in

vivo determination of intermediates in advanced Maillard reaction. *Biochem Biophys Res Commun* 256:89–93, 1999
14. Thornalley PJ: Advanced glycation and development of diabetic complications: Unifying the involvement of glucose, methylglyoxal and oxidative stress. *Endocrinol Metab* 3:149–166, 1996
15. Brownlee M, Vlassara H, Kooney, *et al*: Aminoguanidine prevents diabetes induced arterial wall protein cross-linking. *Science* 232:1629–1632, 1986
16. Miyata T, Ueda Y, Asahi K, *et al*: Mechanism of the inhibitory effect of OPB-9195 [(±)-2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide] on advanced glycataion end products and advanced lipoxidation end products formation. *J Am Soc Nephrol* 11:1719–1725, 2000
17. Miyata T, Ueda Y, Shinzato T, *et al*: Accumulation of albumin-linked and free-form pentosidine in the circulation of uremic patients with end-stage renal failure: Renal implications in the pathophysiology of pentosidine. *J Am Soc Nephrol* 7:1198–1206, 1996
18. Miyata T, Fu MX, Kurokawa K, *et al*: Autoxidation products of both carbohydrates and lipids are increased in uremic plasma: Is there oxidative stress in uremia? *Kidney Int* 54:1290–1295, 1998
19. Fu MX, Requena JR, Jenkins AJ, *et al*: The advanced glycation end product, N^{ε}-(carboxymethyl) lysine, is a product of lipid peroxidation and glycoxidation reactions. *J Biol Chem* 271:9982–9986, 1996
20. McLellan AC, Thornalley PJ: Glyoxalase activity in human red blood cells fractioned by age. *Mech Ageing Dev* 48:63–71, 1989
21. Ranganathan S, Walsh ES, Godwin AK, Tew KD: Cloning and characterization of human colon glyoxalase-I. *J Biol Chem* 268: 5661–5667, 1993
22. Brandt RB, Siegel SA, Waters MG, Bloch MH: Spectrophotometric assay for D-lactate in plasma. *Anal Biochem* 102:39–46, 1980
23. Nishikawa T, Edelstein D, Du XL, *et al*: Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 404:787–790, 2000