Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 58 (2009) 976–982
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Enhanced methylglyoxal formation in the erythrocytes of hemodialyzed patients

Eszter Karg[a,*], Ferenc Papp[a], Noemi Tassi[a], Tamás Janáky[b], Gyula Wittmann[a], Sándor Túri[a]

[a]Department of Pediatrics, University of Szeged, H-6720 Szeged, Hungary
[b]Department of Medical Chemistry, University of Szeged Dóm tér 8, H-6720 Szeged, Hungary

Received 7 October 2008; accepted 23 February 2009

Abstract

Methylglyoxal (MG) contributes significantly to the carbonyl stress in uremia; however, the reason for its increased concentration is not clear. Thus, the present study was aimed to investigate the formation and degradation of MG in the erythrocytes of hemodialyzed (HD) patients with end-stage renal disease. In 22 nondiabetic patients on long-term HD, erythrocyte MG and D-lactate levels, glyoxalase activities, and whole blood reduced glutathione content were determined. The data were compared with those from 22 healthy controls. Erythrocyte MG and D-lactate production were also investigated in vitro under normoglycemic (5 mmol/L) and hyperglycemic (50 mmol/L) conditions. The erythrocyte MG levels were elevated ($P < .001$) in the HD patients. The blood reduced glutathione content and glyoxalase I activity were similar to the control levels, but the glyoxalase II activity was significantly ($P < .005$) increased. In the normoglycemic in vitro model, production of both MG ($P < .001$) and D-lactate ($P < .002$) was significantly enhanced in the HD erythrocytes relative to the controls. During hyperglycemia, the MG formation and degradation rates were further increased ($P < .001$). The present study demonstrated an increased formation of MG in the erythrocytes of HD patients. This seemed to be related to a glucose metabolism disturbance of the cells. The degradation system of MG was also activated; still, it was not able to counteract the high rate of MG formation. The alterations and imbalance of these metabolic processes may contribute to the carbonyl overload and stress in the HD patients.

1. Introduction

The term *carbonyl stress* was proposed for states of generalized increases in reactive carbonyl compounds, such as glyoxal, methylglyoxal (MG), and 3-deoxyglucose, in uremia [1]. The reactions between these compounds and proteins result in the formation of protein cross-links (advanced glycation end products [AGEs]), this being associated with enzyme inactivation, protein denaturation, and cellular immune response. Their reactions with DNA induce mutagenesis and carcinogenesis, whereas the formation of lipid adducts causes membrane lipid bilayer disruption. Carbonyl stress appears to participate in the pathogenesis of long-term complications associated with end-stage renal disease (ESRD) and dialysis, such as accelerated atherosclerosis, enhanced cancer development, and amyloidosis [2,3].

The importance of the carbonyl compound MG in uremia is increasingly recognized. Elevated levels of MG-derived AGEs and MG itself are found in the plasma of ESRD patients [4,5]. The level of the MG-derived lysine dimer (MOLD) is significantly elevated in the sera of nondiabetic uremic patients where, together with the glyoxal-derived lysine dimer (GOLD), it forms the major class of plasma protein cross-links [6]. However, the reason for the increased concentration of MG in uremia is still not clear.

The uremic state and hemodialysis (HD) are associated with enhanced oxidative stress, which has been suggested to play a role in both the production and catabolism of carbonyl compounds [7]. Methylglyoxal may be formed in the oxidative or nonoxidative metabolism of carbohydrates, lipids, or amino acids; but its main source is considered to be the nonoxidative, spontaneous transformation of triose phosphates [8]. Oxidative stress decreases the cellular concentration of reduced glutathione (GSH), a cofactor for

* Corresponding author. Department of Pediatrics, Albert Szent-Györgyi Medical School, University of Szeged, H-6720 Szeged, Hungary. Tel.: +36 62 545331; fax: +36 62 545329.
E-mail address: karg@pedia.szote.u-szeged.hu (E. Karg).

0026-0495/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.02.032

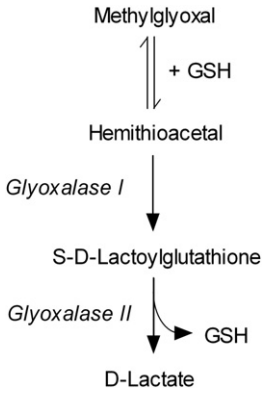

Fig. 1. The glyoxalase pathway.

the detoxification of MG by the glyoxalases (Fig. 1). Experimental GSH depletion via an oxidative or nonoxidative mechanism induced a marked accumulation of MG [9]. In mouse lenses, however, even at approximately 80% GSH loss after buthionine sulfoximine treatment, the accumulation of MG was not enhanced [10]. Furthermore, a decreased GSH level in the blood of uremic patients is not a general finding [11,12].

In 1 uremic subject, low glyoxalase I activity was accompanied by augmented levels of carbonyl compounds [13]. However, this was a unique case; the glyoxalase I activity of a group of patients with ESRD in the same study proved normal.

The aim of the present study was to characterize the MG metabolism in the erythrocytes of HD patients with ESRD. We report enhanced formation and degradation of MG in vitro in the cells, and also observations concordant with an elevated MG turnover in vivo in the erythrocytes. The enhanced rate of formation overwhelmed the rate of degradation of the carbonyl compound. The alterations and imbalance of these metabolic processes may contribute to the MG overload and carbonyl stress in HD patients.

2. Patients and methods

2.1. Patients

Twenty-two nondiabetic patients (9 male, 13 female; age, 42 ± 24 [mean ± SD]) on long-term HD were included. Their original nephrologic diagnoses were chronic pyelonephritis with reflux nephropathy, interstitial nephritis, membranoproliferative glomerulonephritis, hypoplastic kidney, and polycystic kidney. The patients were on 4-hour bicarbonate HD performed on a Polyflux 17L dialyzer (Gambro Dialysatoren GmBH, Hechingen, Germany) 3 times a week. The median time spent on the dialysis program was 40 months (quartiles 20 and 76 months). Blood samples were collected in a fasting state before the first dialysis session of the week. The control group comprised 22 healthy adult volunteers (9 male, 13 female; age, 43 ± 12 years [mean ± SD]).

The study was approved by the Human Investigation Review Board of the University of Szeged, and informed consent was obtained before the collection of blood.

2.2. Methylglyoxal

Methylglyoxal was determined by electrospray tandem mass spectrometry. Venous blood anticoagulated with EDTA was separated by centrifugation (4°C, 1500*g*, 10 minutes). Plasma and erythrocytes washed in phosphate-buffered saline (pH 7.4) were deproteinized with equal volumes of 24% trifluoroacetic acid (TFA). Internal standardization was achieved by addition of 2,3-hexanedione in a final concentration of 200 nmol/L to the samples [14]. The deproteinized extracts were derivatized with 100 μmol/L 1,2-diaminobenzene in the presence of 100 μmol/L diethylenetriaminepentaacetic acid, and 1.2 mmol/L sodium dithionite at room temperature for 4 hours in the dark [15]. The derivatization of MG and hexanedione with 1,2-diaminobenzene yields 2-methylquinoxaline and 1-methyl-2-propylquinoxaline, respectively. The samples were extracted with 4 vol of chloroform [16]; and after evaporation, the residue was dissolved in 100 μL of methanol/water/TFA (50:50:0.1, vol/vol/vol) solution.

The mobile phase (methanol/water, 50:50 vol/vol) was delivered by Waters Separation Module 2795 (Waters, Manchester, United Kingdom) at a flow rate of 200 μL/min. The samples (20 μL) were analyzed directly by positive ion electrospray tandem mass spectrometry (Micromass Quattro Micro, Waters) in multiple reaction monitoring mode. To improve the assay specificity, 2 mass transitions were detected for MG: *m/z* 144.8 to 76.2 d and 144.8 to 117.6 d. Hexanedione was analyzed with the mass transition *m/z* 157.8 to 89.0 d. The ionization source temperature was 120°C, and the desolvation gas temperature was 350°C. The cone gas and the desolvation gas flow rates were 150 and 550 L/h, respectively. The capillary voltage was 3.5 kV, and the cone voltage was 40 V. Argon gas (2.0×10^{-3} mbar) was in the collision cell, and the collision voltage was 28 eV.

The sensitivity for the mass transition *m/z* 144.8 to 76.2 d was approximately 4-fold higher than that for *m/z* 144.8 to 117.6 d, but the values obtained with the 2 methods correlated well ($r = 0.9982$). The intra- and interassay variations (100 nmol/L) were 10.3% and 12.2% for *m/z* 144.8 to 76.2 d and 11.8% and 12.7% for *m/z* 144.8 to 117.6 d, respectively.

For calibration, MG (in final concentrations of 50-1000 nmol/L) was added to pooled plasma samples and processed as described above. The response curve proved linear in the tested range.

2.3. D-Lactate

D-Lactate was measured by end-point enzymatic assay with D-lactate dehydrogenase in the presence of sodium L-glutamate and glutamate pyruvate transaminase [17]. The increase in reduced nicotinamide adenine dinucleotide (NADH) concentration was detected fluorometrically.

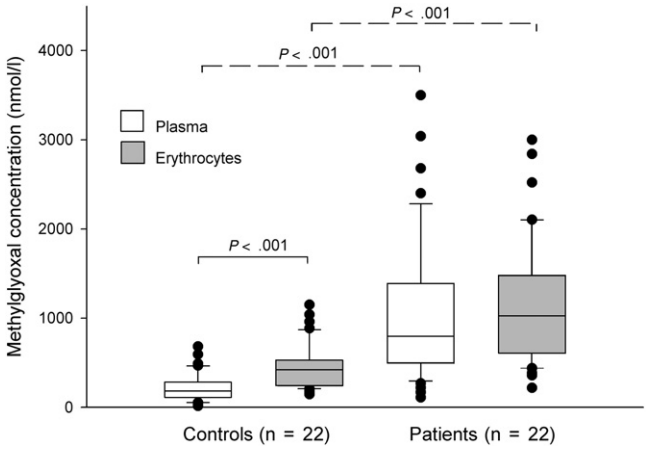

Fig. 2. Methylglyoxal levels in plasma and erythrocytes of controls and HD patients.

2.4. Glyoxalase I and II

The glyoxalase I activity in the erythrocytes was assayed by measuring the initial rate of formation of *S*-D-lactoylglutathione from its hemithioacetal in the presence of diluted erythrocyte lysate as described by McLellan and Thornalley [18]. The glyoxalase II activity was assayed by measuring the initial rate of hydrolysis of *S*-D-lactoylglutathione. The reactions were monitored spectrophotometrically at 240 nm.

2.5. Reduced glutathione

Sensitive and specific determination of erythrocyte GSH concentration was made by an enzymatic method [19]. The combined action of 5,5′dithio-bis(2-nitrobenzoic acid) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of glutathione reductase results in a reaction cycle, the rate of which depends on the concentration of total (oxidized +reduced) glutathione content recorded spectrophotometrically at 412 nm during the first 6 minutes. Oxidized glutathione was determined separately after alkylation of GSH with *N*-ethylmaleimide and separation by gel filtration. The GSH levels were expressed with reference to erythrocyte volume.

2.6. Isolation and incubation of human erythrocytes

Human erythrocytes were isolated and tested in vitro by the method of Thornalley et al [16]. Briefly, venous blood anticoagulated with EDTA was centrifuged (2000*g*, 5 minutes); and the plasma and white blood cells were removed. The packed erythrocyte pellet was washed 3 times with 4 vol of phosphate-buffered saline (pH 7.4) and washed a fourth and final time with 4 vol of Krebs-Ringer phosphate buffer (pH 7.4). Cells (50%, vol/vol) were incubated in Krebs-Ringer phosphate buffer with 5 or 50 mmol/L glucose for 2 hours at 37°C. In samples including 50 mmol/L glucose, isotonicity was preserved by decreasing the concentration of NaCl.

After incubation, the erythrocyte suspensions were deproteinized with equal volumes of 24% TFA. Zero time samples were 50% erythrocyte suspensions inactivated by ice-cold TFA before the addition of glucose. The deproteinized extracts were processed for MG and D-lactate analyses as described above.

2.7. Statistical analysis

Depending on the distribution of the data, both parametric (Student *t* test or analysis of variance followed by the Tukey test) and nonparametric (Mann-Whitney test) methods were used for the statistical analyses. In all cases, *P* values less than .05 were considered statistically significant.

3. Results

3.1. Methylglyoxal

The MG concentration was significantly elevated in both the erythrocytes and plasma of the HD patients relative to the respective control values ($P < .001$ for both) (Fig. 2). In the controls, the MG level was significantly higher in the erythrocytes than in the plasma ($P < .001$) (Fig. 2); but in the HD patients, there was no significant difference between the 2 compartments.

3.2. Glyoxalase I and II

There was no significant difference in glyoxalase I activity, but the glyoxalase II activity proved elevated ($P < .005$) in the HD patients (Fig. 3).

3.3. Reduced glutathione

The erythrocyte GSH level did not differ significantly in the HD patients and the controls (1.95 ± 0.45 vs 2.12 ± 0.68 mmol/L [mean ± SD]).

3.4. D-Lactate

There was a nonsignificant rise in the erythrocyte D-lactate level in the HD patients, but a marked ($P < .05$) decrease in the

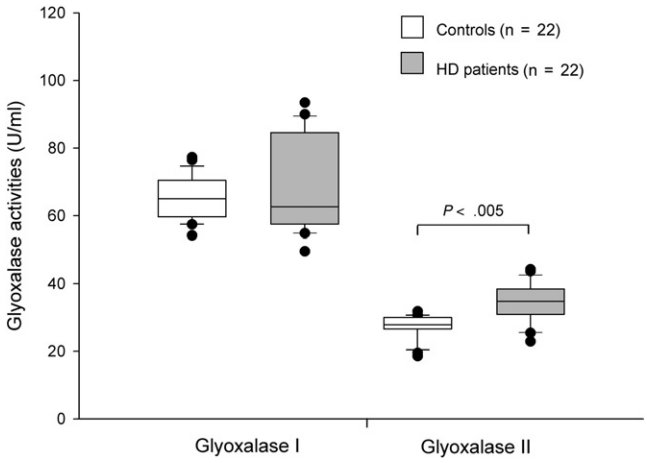

Fig. 3. Glyoxalase activities in erythrocytes of controls and HD patients.

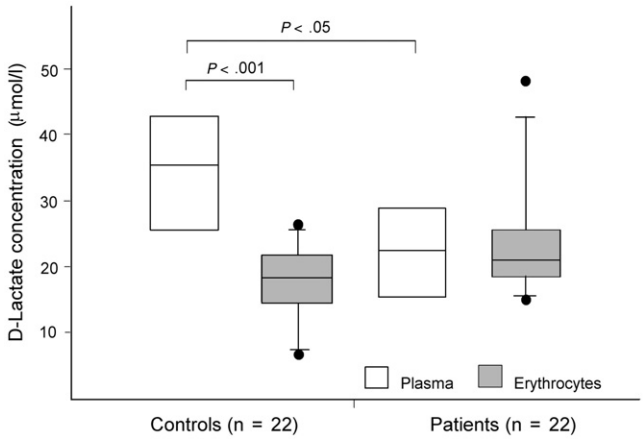

Fig. 4. D-Lactate levels in plasma and erythrocytes of controls and HD patients.

plasma compared with the controls (Fig. 4). In the controls, the D-lactate level was significantly higher ($P < .001$) in the plasma than in the erythrocytes, whereas in the patients, there was no difference between the 2 compartments.

3.5. Methylglyoxal and D-lactate production in vitro in erythrocytes under normoglycemic and hyperglycemic conditions

The MG concentration was significantly (0 h: $P < 040$; 2 h: $P < 001$) increased in the normoglycemic (5 mmol/L) HD erythrocyte suspensions (Fig. 5A). Incubation of erythrocytes with 50 mmol/L glucose for 2 hours caused the MG levels to increase approximately 8- to 12-fold relative to the normoglycemic model in both groups ($P < .001$ for both).

Both normoglycemic and hyperglycemic conditions induced significantly ($P < .002$ and $P < .001$, respectively) higher rises in D-lactate level in the HD erythrocyte suspensions (Fig. 5B). The median D-lactate production rates were 16.1 vs 12.9 µmol/h (5 mmol/L glucose) and 164.4 vs 125.3 µmol/h (50 mmol/L glucose), respectively.

4. Discussion

The mechanism underlying the increased presence of the AGE precursor molecule MG in uremia is not clear. The MG overload may be due either to increased formation or to decreased clearance or degradation. Impaired carbonyl compound clearance has been suggested to contribute to the carbonyl stress in uremic patients [20]. Upon dialysis, the MG and glyoxal levels were shown to decrease in the plasma, but were still higher than those in the controls [21].

The present study also demonstrated elevated MG concentrations in the erythrocytes of HD patients, suggesting an increased formation or impaired degradation of the compound.

Indeed, the normoglycemic in vitro model revealed enhanced formation of MG in the HD erythrocytes, indicated by the elevated MG plus D-lactate levels. Hyperglycemia increased the rate of MG formation in both the HD and control erythrocytes approximately 10-fold, the rise having been higher in the erythrocytes of the patients. The differences observed in the presence of equal concentrations of glucose suggest a glucose metabolism disturbance in the HD erythrocytes.

Methylglyoxal is an unavoidable product of the glycolytic pathway. The spontaneous, nonenzymatic degradation of the glycolytic intermediates dihydroxyacetone phosphate and glyceraldehyde-3-phosphate (GA3P) is considered to be its major source [8]. Enhanced MG formation may occur because of an elevated glycolytic rate or because of enzyme defects distal to the triose phosphates dihydroxyacetone phosphate and GA3P. Triose phosphate accumulation is also influenced by the activity of the pentose phosphate pathway (PPP): the conversion of GA3P to ribose-5-phosphate diminishes the pool of MG precursors [16].

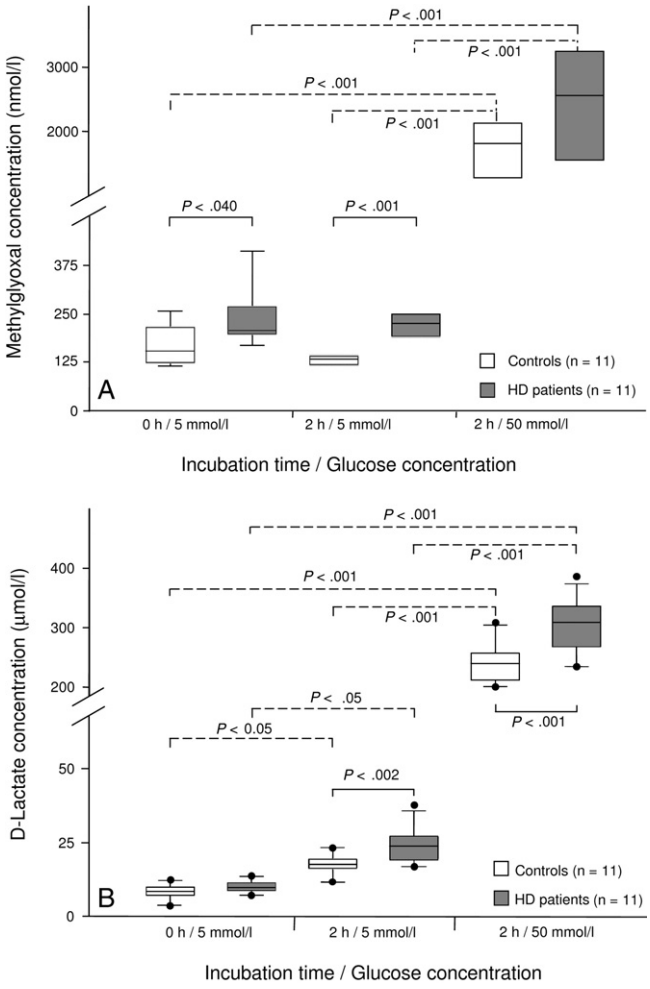

Fig. 5. Methylglyoxal (A) and D-lactate (B) levels in erythrocyte suspensions (50% vol/voL) from controls and HD patients at zero time and after incubation for 2 hours at 37°C under normoglycemic (5 mmol/L) and hyperglycemic (50 mmol/L) conditions.

Patients with ESRD are known to have a complex disturbance of the carbohydrate metabolism including insulin resistance [22], hyperglucagonemia [23], impaired glucose production, and nonoxidative glucose disposal [24,25]. Glucose uptake and oxidation are probably also affected in the peripheral tissues (muscle, liver, erythrocytes), although the observations are controversial: diminished, enhanced, and normal rates have all been reported [26-31]. Furthermore, inefficient phosphoglyceromutase activity diminishing the activity of PPP occurred in the erythrocytes of uremic patients [31]. The activity of the PPP enzyme transketolase was likewise lower [32], although it was found to normalize upon erythropoietin therapy [33].

The increased D-lactate production observed in the in vitro models in the present study indicated not only an enhanced formation, but also an increased degradation of MG in the erythrocytes of HD patients relative to the controls. In the hyperglycemic models, the increase in D-lactate production relative to the respective normoglycemic models proved the large capacity of the degradation systems in both patients and controls. Still, the rate of MG formation overwhelmed the rate of its degradation, which became more pronounced in the hyperglycemic models of both groups.

Several enzymes may contribute to the catabolism of MG: aldose reductase, the glyoxalases, betaine-aldehyde dehydrogenase, and 2-oxoaldehyde dehydrogenase [34]. Out of these, the principal route for detoxification is provided by the ubiquitous glyoxalase system. It comprises 2 enzymes, glyoxalase I and II, and a catalytic amount of GSH (Fig. 1). The fate of greater than 99% of the MG formed in the erythrocytes is metabolism by this pathway to D-lactate, which is not further metabolized in the erythrocytes [35].

In the present study, the in vivo GSH pool and glyoxalase I activity in the HD patients were similar to those in the controls, whereas the activity of glyoxalase II was significantly enhanced. The erythrocytes in HD patients are much younger than those in healthy individuals because of an increased turnover rate. Glyoxalase I and II activities significantly increase during the maturation of erythrocytes, except in the most dense, old cell fraction [36]. Thus, age-related changes cannot account for the enhancement of glyoxalase II activity observed in the erythrocytes of HD patients.

The rate-limiting step of the glyoxalase pathway is the catalysis by glyoxalase II [37]. If the MG influx exceeds the glyoxalase II activity, the system consumes GSH; and the MG concentration increases. Incubation of erythrocytes with MG induces an initial, extremely rapid fall in GSH that is rapidly restored in healthy subjects, but is substantially delayed in glyoxalase II deficiency [38]. In differentiating HL60 promyelocytes, with the decrease in the activity of glyoxalase I and the increase in the activity of glyoxalase II, there was a concomitant decrease in cellular MG and *S*-D-lactoylglutathione concentrations, indicating a major controlling influence of the activity of glyoxalase II [39]. Furthermore, overexpression of GLX2, which encodes glyoxalase II, inhibits the apoptotic cellular response to MG [40].

The enhanced glyoxalase II activity observed in the present study is possibly due to induction of the enzyme in erythrocyte precursors by the elevated MG level and may be in accordance with the enhanced formation and degradation of MG. In a previous study on patients with triose phosphate isomerase deficiency, the increased metabolism of MG (indicated by a high D-lactate concentration) was proceeded with normal glyoxalase I but increased glyoxalase II activities [41]. The induction of both enzymes does not seem to be a prerequisite for the management of a higher flux through the glyoxalase pathway as long as the activity of glyoxalase I is not overwhelmed.

In the present study, however, the elevated intracellular MG level and the enhanced activity of glyoxalase II were not accompanied in vivo by D-lactate accumulation in the erythrocytes. Indeed, in the plasma of HD patients, significantly decreased D-lactate levels were observed. Previously, the serum D-lactate level has been reported to be similar in HD patients and a small group (n = 4) of controls [13].

In the in vitro erythrocyte models, D-lactate can accumulate because of the lack of clearance and further metabolism. However, D-lactate is not an end product in vivo [42]. After the infusion of DL-lactate into humans, approximately 90% of the D-lactate was metabolized; and only 10% was excreted in the urine [43].

Hepatocytes can take up D-lactate formed by other tissues from the bloodstream [44]. Three different translocators exist to transport D-lactate into the mitochondria of hepatocytes, where it is oxidized to pyruvate by D-lactate dehydrogenase [45]. Pyruvate may be transformed to oxalacetic acid and malate, efflux of which to the cytosol enables gluconeogenesis [46]. The identification and characterization of putative human D-lactate dehydrogenase have been described [47]. Furthermore, oxidation to CO_2 and gluconeogenesis from D-lactate have been reported [46,48].

We presume that the D-lactate produced in vivo in the erythrocytes of HD patients by the enhanced formation and degradation of MG is transferred into the hepatocytes where it is oxidized and probably enters gluconeogenesis.

In summary, the novel findings of this study (the elevated MG level and glyoxalase II activity in the HD erythrocytes, and the increased MG and D-lactate levels in the normoglycemic and hyperglycemic in vitro models) demonstrated an enhanced formation of MG in the erythrocytes of HD patients. The in vitro observations suggested that it was most probably associated with a glucose metabolism disturbance of the cells. The degradation of MG was also enhanced; still, it was not able to counteract the high rate of MG formation.

The altered glucose metabolism of the erythrocytes, which may play an important role in the carbonyl overload and stress in HD patients, needs further elucidation.

Acknowledgment

We thank the patients and stuff of the Dialysis Unit and the controls for their participation in the study. We wish to thank Ilona Szecsi for her excellent technical assistance. The study was supported by National Scientific Research Foundation Hungary (OTKA) grant T 46921.

References

[1] Miyata T, Ueda Y, Yamada Y, et al. Accumulation of carbonyls accelerates the formation of pentosidine, an advanced glycation end product: carbonyl stress in uremia. J Am Soc Nephrol 1998;9: 2349-56.

[2] Miyata T, van Ypersele de Strihou C, Kurokawa K, Baynes JW. Alterations in nonenzymatic biochemistry in uremia: origin and significance of "carbonyl stress" in long-term uremic complications. Kidney Int 1999;55:389-99.

[3] Stopper H, Schinzel R, Sebekova K, Heidland A. Genotoxicity of advanced glycation end products in mammalian cells. Cancer Lett 2003;190:151-6.

[4] Odani H, Shinzato T, Matsumuto Y, Usami J, Maeda K. Increase in three alpha,beta-dicarbonyl compound levels in human uremic plasma: specific in vivo determination of intermediates in advanced Maillard reaction. Biochem Biophys Res Commun 1999;256:89-93.

[5] Degenhardt TP, Thorpe SR, Baynes JW. Chemical modification of proteins by methylglyoxal. Cell Mol Biol (Noisy-le-grand) 1998;44: 1139-45.

[6] Odani H, Shinzato T, Matsumuto Y, Brinkmann Frye E, Baynes JW, Maeda K. Imidazolium crosslinks derived from reaction of lysine with glyoxal and methylglyoxal are increased in serum proteins of uremic patients: evidence for increased oxidative stress in uremia. FEBS Lett 1998;427:381-5.

[7] Miyata T, Wada Y, Caiu 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 1997;51:1170-81.

[8] Thornalley PJ. The glyoxalase system: new developments towards functional characterization of a metabolic pathway fundamental to biological life. Biochem J 1990;269:1-11.

[9] Thornalley PJ. Glyoxalase I—structure, function and a critical role in the enzymatic defence against glycation. Biochem Soc Trans 2003;31: 1343-8.

[10] Staniszewska MM, Nagaraj RH. Upregulation of glyoxalase I fails to normalize methylglyoxal levels: a possible mechanism for biochemical changes in diabetic mouse lenses. Mol Cell Biochem 2006;288: 29-36.

[11] Klemm A, Voigt C, Friedrich M, Funfstuck R, et al. Determination of erythrocyte antioxidant capacity in haemodialysis patients using electron paramagnetic resonance. Nephrol Dial Transplant 2001;16: 2166-71.

[12] Suliman ME, Divino Filho JC, Barany P, Anderstam B, Lindholm B, Bergstrom J. Effects of high-dose folic acid and pyridoxine on plasma and erythrocyte sulfur amino acids in hemodialysis patients. J Am Soc Nephrol 1999;10:1287-96.

[13] Miyata T, van Ypersele de Strihou C, Imasawa T, et al. Glyoxalase I deficiency is associated with an unusual level of advanced glycation end products in a hemodialysis patient. Kidney Int 2001;60:2351-9.

[14] Randell EW, Vasdev S, Gill V. Measurement of methylglyoxal in rat tissues by electrospray ionization mass spectrometry and liquid chromatography. J Pharmacol Toxicol Methods 2005;51:153-7.

[15] Dobler D, Ahmed N, Song L, Eboigbodin KE, Thornalley PJ. Increased dicarbonyl metabolism in endothelial cells in hyperglycemia induces anoikis and impairs angiogenesis by RGD and GFOGER motif modification. Diabetes 2006;55:1961-9.

[16] Thornalley PJ, Jahan I, Ng R. Suppression of the accumulation of triosephosphates and increased formation of methylglyoxal in human red blood cells during hyperglycaemia by thiamine in vitro. J Biochem 2001;129:543-9.

[17] Anderson YS, Curtis NJ, Hobbs JAR, et al. High serum D-lactate in patients on continuous ambulatory peritoneal dialysis. Nephrol Dial Transplant 1997;12:981-3.

[18] McLellan AC, Thornalley PJ. Sample storage conditions for the assay of glyoxalase activities in whole blood samples. Ann Clin Biochem 1992;29:222-3.

[19] Nemeth I, Boda D. Blood glutathione redox ratio as a parameter of oxidative stress in premature infants with IRDS. Free Radic Biol Med 1994;16:347-53.

[20] 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 2000;11:1744-52.

[21] Lapolla A, Flamini R, Lupo A, et al. Evaluation of glyoxal and methylglyoxal levels in uremic patients under peritoneal dialysis. Ann N Y Acad Sci 2005;1043:217-24.

[22] DeFronzo RA, Alvestrand A, Smith D, Hendler R, Hendler E, Wahren J. Insulin resistance in uremia. J Clin Invest 1981;67:563-8.

[23] Bilbrey GL, Faloona GR, White MG, Knochel JP. Hyperglucagonemia of renal failure. J Clin Invest 1974;53:841-7.

[24] Rubenfeld S, Garber AJ. Abnormal carbohydrate metabolism in chronic renal failure. The potential role of accelerated glucose production, increased gluconeogenesis, and impaired glucose disposal. J Clin Invest 1978;62:20-8.

[25] Maillet C, Garber AJ. Skeletal muscle amino acid metabolism in chronic uremia. Am J Clin Nutr 1980;33:1343-53.

[26] Weisinger JR, Contreras NE, Cajias J, Bellorin-Font E, Amair P, Guitierrez L, et al. Insulin binding and glycolytic activity in erythrocytes from dialyzed and nondialyzed uremic patients. Nephron 1988;48:190-6.

[27] Wann JG, Lin CS, Chang LC, et al. Enhanced expression of glucose transporter 1 on erythrocyte membrane in hemodialysis patients: the possible role in erythrocyte ascorbate recycling. Am J Kidney Dis 2006;47:1055-63.

[28] Foss MC, Gouveia LM, Moyses Neto M, Paccola GM, Piccinato CE. Effect of hemodialysis on peripheral glucose metabolism of patients with chronic renal failure. Nephron 1996;73:48-53.

[29] Quintanilla A, Shambaugh III GE, Gibson TP, Craig R. Glucose metabolism in uremia. Am J Clin Nutr 1980;33:1446-50.

[30] Rigalleau V, Gin H. Carbohydrate metabolism in uraemia. Curr Opin Clin Nutr Metab Care 2005;8:463-9.

[31] Yawata Y, Jacobs HS. Abnormal red cell metabolism in patients with chronic uremia: nature of the defect and its persistence despite adequate hemodialysis. Blood 1975;45:231-9.

[32] Lonergan ET, Semar M, Sterzel RB, et al. Erythrocyte transketolase activity in dialyzed patients. A reversible metabolic lesion of uremia. N Engl J Med 1971;284:1399-403.

[33] Taccone-Galluci M, Lubrano R, Trapasso E, et al. Oxidative damage to RBC membranes and pentose phosphate shunt activity in hemodialysis patients after suspension of erythropoietin treatment. ASAIO J 1994; 40:M663-6.

[34] Vander Jagt DL, Hundsaker LA. Methylglyoxal metabolism and diabetic complications: roles of aldose reductase, glyoxalase-I, betaine aldehyde dehydrogenase and 2-oxoaldehyde dehydrogenase. Chem Biol Interact 2003;143-144:341-51.

[35] Martins AM, Mendes P, Cordeiro C, Freire AP. In situ kinetic analysis of glyoxalase I and glyoxalase II in Saccharomyces cerevisiae. Eur J Biochem 2001;268:3930-6.

[36] McLellan AC, Thornalley PJ. Glyoxalase activity in human red blood cells fractioned by age. Mech Ageing Dev 1989;48:63-71.

[37] Breborowicz A, Witowski J, Polubinska A, Pyda M, Oreopulos D. L-2-oxothiazolidine-4-carboxylic acid reduces in vitro cytotoxicity of glucose degradation products. Nephrol Dial Transplant 2004;19:3005-11.

[38] Valentine WN, Paglia DE, Neerhout RC, Konrad PN. Erythrocyte glyoxalase II deficiency with coincidental hereditary elliptocytosis. Blood 1970;36:797-808.

[39] Hooper NI, Tisdale MJ, Thornalley PJ. Modification of the glyoxalase system in human HL60 promyelocytic leukaemia cells during differentiation to neutrophils in vitro. Biochim Biophys Acta 1988; 966:362-9.

[40] Xu Y, Chen X. Glyoxalase II, a detoxifying enzyme of glycolysis byproduct methylglyoxal and a target of p63 and p73, is a pro-survival factor of the p53 family. J Biol Chem 2006;281: 26702-13.

[41] Karg E, Nemeth I, Horanyi M, Pinter S, Vecsei L, Hollan S. Diminished blood levels of reduced glutathione and alpha-tocopherol in two triosephosphate isomerase-deficient brothers. Blood Cells Mol Dis 2000;26:91-100.

[42] Ewaschuk JB, Naylor JM, Zello GA. D-lactate in human and ruminant metabolism. J Nutr 2005;135:1619-25.

[43] Oh MS, Uribarri J, Alveranga D, Lazar I, Bazilinski N, Carrol JH. Metabolic utilization and renal handling of D-lactate in men. Metabolism 1985;34:621-5.

[44] Fafoumoux P, Demigne C, Remesy C. Carrier-mediated uptake of lactate in rat hepatocytes. Effects of pH and possible mechanisms for L-lactate transport. J Biol Chem 1985;260:292-9.

[45] de Bari L, Atlante A, Guaragnella N, Principato G, Passarella S. D-Lactate transport and metabolism in rat liver mitochondria. Biochem J 2002;365:391-403.

[46] Harmon DL, Britton RA, Prior RL. In vitro rates of oxidation and gluconeogenesis from L(+)- and D(−)lactate in bovine tissues. Comp Biochem Physiol B 1984;77:365-8.

[47] Flick MJ, Konieczny SF. Identification of putative mammalian D-lactate dehydrogenase enzymes. Biochem Biophys Res Commun 2002;295: 910-6.

[48] Brandt RB, Waters MG, Rispler MJ, Kline ES. D- and L-lactate catabolism to CO2 in rat tissues. Proc Soc Exp Biol Med 1984;175:328-35.