Journal of Chromatography B, 879 (2011) 3214–3219

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Determination of time-dependent accumulation of D-lactate in the streptozotocin-induced diabetic rat kidney by column-switching HPLC with fluorescence detection☆

Mei-Hsiang Lin[a], Hsiang-Yin Chen[a], Tzu-Hsin Liao[a], Tzu-Chuan Huang[a], Chien-Ming Chen[b], Jen-Ai Lee[a,*]

[a] School of Pharmacy, College of Pharmacy, Taipei Medical University, 250 Wuxing St., Taipei 11031, Taiwan
[b] Department of Electro-Optical Engineering, National Taipei University of Technology, No. 1, Sec. 3, Chung-Hsiao E. Rd., Taipei 106, Taiwan

ARTICLE INFO

Article history:
Received 30 November 2010
Accepted 8 February 2011
Available online 13 February 2011

Keywords:
D-Lactate
L-Lactate
Diabetic rat
Nephropathy
Streptozotocin
Methylglyoxal

ABSTRACT

For better understanding the complete metabolism and the physiological role of D-lactate, the concentrations of D-lactate in the serum, liver and kidney of normal and diabetic rats were determined by our established column-switching HPLC method with pre-column fluorescence derivatization. Eight-week-old male Sprague–Dawley rats were administered with streptozotocin (STZ) (80 mg/kg) or citrate buffer intraperitoneally. The tissues were then removed and homogenized after 4, 8, 12 and 16 weeks of drug administration, respectively. The homogenates were centrifuged at 1200 × *g* for 10 min, then the supernatants were derivatized with a fluorescent reagent, 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBD-PZ), separated on an ODS column followed by a Chiralpak AD-RH chiral column for enantioseparation. The results showed that the D-lactate content elevated in all the 3 examined tissues under diabetic stages. In addition, D-lactate concentrations in rat kidney were accumulated significantly and time-dependently in diabetic groups after receiving STZ for 4, 8, 12 and 16 weeks (2.99, 13.11, 18.19, 23.23 vs. 0.79 μmol/mg protein as control group). Moreover, the kidney of induced 12-week diabetic rat renal showed some histological changes of progressive diabetic nephropathy. The results suggest that D-lactate may be used as a marker of diabetic nephropathy.

1. Introduction

The D-lactate, an endogenous product from excessive use of regular energy resources, has been tested to use as a marker of several diseases. It has reported to increase in bacterial infection [1,2], acute intestinal ischemia [3–6], and appendicitis [7,8]. The most recent application is on its relationship with the severity of diabetics. Significant increases of serum, plasma and urinary D-lactate under diabetic stage were found in our previous publications [9–11]. In diabetic stage, with the catalysis by glyoxalase I and II being the major producing pathway [12,13], the plasma level of D-lactate was found to elevate proportionally with the increasing production of its precursor, methylglyoxal (MG), in plasma [14–17]. However, the concentrations of D-lactate and MG in other biological samples at normal and diabetic stage are warranted to determine for establishing the model of application in diabetes.

Measuring the concentrations of D-lactate in biological samples encounters many barriers. Lactic acid (2-hydroxypropionic acid), the chemical formula of $C_3H_6O_3$, has a chiral center to form two enantiomers, L-(+)-lactic acid and D-(−)-lactic acid. With a pK_a value of 3.86, lactic acid mostly dissolves to form lactate in the physiological fluids. The automated metabolite analyzers can only determine the content of L-lactate by using L-lactate dehydrogenase. And the traditional enzymatic method by D-lactate dehydrogenase (D-LDH), although widely used to measure D-lactate in biological samples, cross reacts with many endogenous substances, such as pyruvate, *S*-lactonyl glutathione, L-lactate, fructose 1,6-bisphosphate, and 3-phosphoglyceric acid, reducing its accuracy and precision significantly [18,19]. To overcome the above drawbacks, many high-performance liquid chromatography (HPLC) methods have been developed and modified by our previous efforts [9,20]. We previously determined the D-lactate in urine of normal and diabetic rats by HPLC with an octadecylsilica (ODS) column connected to an amylose-based chiral column.

Abbreviations: DM, diabetes mellitus; TFA, trifluoroacetic acid; NBD-PZ, 4-nitro-7-piperazino-2,1,3-benzoxadiazole; I.S., internal standard; *o*-PD, *o*-phenylenediamine; 2-MQ, 2-methylquinoxaline; 5-MQ, 5-methylquinoxaline; AGEs, advanced glycation end products.

☆ This paper is part of the special issue "Analysis and Biological Relevance of D-Amino Acids and Related Compounds", Kenji Hamase (Guest Editor).

* Corresponding author. Tel.: +886 2 2736 1661x6125; fax: +886 2 2736 1661x6120.
E-mail address: jenai@tmu.edu.tw (J.-A. Lee).

1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2011.02.015

This method allows precisely measuring the urinary D-lactate at a detection limit of 10 nM, the accuracy between 96.93–104.85%, and the intra- and inter-day precision of 0.80 and 14.44%, respectively. These improvements make the clinical application of D-lactate in diabetics a step towards to be possible.

Before using D-lactate as an early marker to stage the severity of diabetics or nephropathy, the distribution of D-lactate in the normal and diabetic subjects should be further studied. In order to investigate the alteration of D-lactate levels in circular pools and the tissues responsible for gluconeogenesis in diabetic rats, the content of D-lactate and L-lactate in the kidney, liver and also serum of diabetic and normal rats were determined simultaneously, after 4, 8, 12 and 16 weeks of diabetic induction. In addition, the histological examination of kidney in normal and diabetic rat and the level of MG were also studied.

2. Materials and methods

2.1. Chemicals

The fluorescence reagent, 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBD-PZ), and condensing reagents, triphenylphosphine (TPP) and 2,2′-dipyridyl disulfide (DPDS) were from Tokyo Kasei Chemicals (Tokyo, Japan). Lithium D- and L-lactate, streptozotocin, formalin, 2-methylquinoxaline (2-MQ), 5-methylquinoxaline (5-MQ), and *o*-phenylenediamine (*o*-PD) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Propionic acid, citric acid, and sodium hydroxide were purchased from Nacalai Tesque (Tokyo, Japan). Trifluoroacetic acid (TFA) was obtained from Riedel-de Haën (Seelze, Germany). Methanol (MeOH) and acetonitrile (CH_3CN) were of HPLC grade from Merck (Darmstadt, Germany). All the reagents were analytical grade.

2.2. Animals

Eight-week-old male Sprague–Dawley rats were used in this study. The experimental protocol was approved by the Animal Care and Use Committee of Taipei Medical University, Taiwan. The animals were housed three per cage under a condition of a 12-h light/dark cycle, 25 °C temperature, and given standard lab chow and water ad libitum. After one week of acclimatization, the rats were administered intraperitoneally either STZ (80 mg/kg) in citrate buffer (50 mM, pH 4.5) to induce diabetics, or citrate buffer alone to be the normal controls [21]. STZ was employed as an agent to induce the Type I diabetes mellitus via causing the death of insulin-producing β cells in the islets of Langerhans according to the previous literature [22,23]. With a structure similar to glucose, STZ can be uptake into the β cells by the glucose transport protein GLUT2, causing toxic damage to the DNA of the cells [24]. Body weights and blood glucose of diabetic and normal rats were compared to ensure the successful induction of diabetics and to monitor the severity of disease. Diabetes mellitus is defined by demonstrating casual plasma glucose higher than 11.1 mmol/L (200 mg/dL) according to World Health Organization [25].

2.3. Glucose concentration

A drop of tail blood was collected to determine the glucose concentration by OneTouch® SureStep® Plus Meter (Lifescan Co. Ltd., California, USA) according to the user's manual.

2.4. Enantiomeric determination of D-lactate in serum, liver, and kidney

After 4, 8, 12 and 16 weeks of STZ or vehicle administration, blood, liver and kidney rapidly removed from anesthetized normal or diabetic rats were collected to homogenize in 2 volume of ice-cold PBS buffer. Homogenates were centrifuged at 4 °C, 14,000 rpm for 10 min (Mikro 22R Centrifuge, Model D-78532, Hettich zentrifugen, Tuttlingen, Germany), and the supernatants were kept at −20 °C until used. Enantiomeric determination of D-lactate was performed by an HPLC system with some modifications according to our previous publication [10]. Briefly, 20 μL of homogenate was mixed with 10 μL of 1 mM propionic acid dissolved in H_2O as internal standard and 170 μL of acetonitrile was added to deprotein. After centrifugation at 4 °C, 14,000 rpm for 10 min, 100 μL of the supernatant was mixed well with 150 μL of 2 mM NBD-PZ, 25 μL each of 280 mM TPP and DPDS in CH_3CN. After standing for 3 h at 30 °C, 250 μL of 0.1% TFA in H_2O was added to stop the derivatization reaction. To remove the excess fluorescent derivatizing reagent, 100 μL of the resultant solution was loaded onto a mobile phase preconditioned Empore™ SBD-RPS (4 mm/1 mL) cartridge and eluted with 200 μL of mobile phase for complete elution, then the total 300 μL of the eluant was collected together. An amount of 20 μL of the eluant was filtered with 0.2-μm filter and subjected to a column-switching HPLC analysis. Protein concentrations of homogenates were assayed according to the method of Braford [26], using the Bio-Rad protein assay kit with BSA as a standard. D-Lactate concentration was expressed as millimole per milligram of protein.

2.5. Column-switching HPLC conditions

D- and L-Lactate were simultaneously quantified by our column-switching HPLC as our previous publications [10,27,28] with some modification. The HPLC system consisted of a Rheodyne injector (Model 7725i) with a 20-μL loop, two Hitachi L-7100 pumps (Tokyo, Japan), a Hitachi F-1000 and an L-7485 fluorescence detector, two integrators (Hitachi D-2500), and a Rheodyne switching valve (Model 7000). A Biosil ODS column (4.6 mm × 250 mm I.D., particle size 5 μm, Biotic Chemical Co., Ltd., Taipei, Taiwan) was utilized for isolation and quantification of the total lactate in rat serum, liver and kidney; the mobile phase was H_2O/MeOH/CH_3CN (70/10/20, v/v/v) at a flow rate of 0.7 mL/min. For the enantiomeric separation of D- and L-lactate, a Chiralpak®AD-RH chiral column (150 mm × 4.6 mm ID., Daicel Chemical Industries Co., Ltd., Osaka, Japan) was used; the mobile phase was H_2O/CH_3CN (60/40, v/v) at a flow rate of 0.3 mL/min. Fluorescence detection was monitored at an excitation wavelength of 491 nm and an emission wavelength of 547 nm. The (D + L)-lactate concentration was calculated in chromatogram obtained from the ODS column, and the proportion of D- or L-lactate was obtained from the chromatogram of AD-RH chiral column. The D-lactate concentration in rat serum, liver and kidney homogenates was determined by the same method.

2.6. Calibration curve study

The calibration curve study was carried out by preparing 1:1 D,L-lactate standard solutions at five concentration levels of 0.05, 0.25, 1.00, 2.00, and 6.00 mM. Ten microliters of 1 mM propionic acid (I.S.) in H_2O and 170 μL of CH_3CN were added to 20 μL of the standard solution, and the same procedures were performed as described above ($n = 6$). The peak area ratio of D,L-lactate to that of I.S. derivative was plotted against the concentrations of D,L-lactic acid. The recovery rate was measured by adding 0.25 mM, 0.5 mM, 1.0 mM D-lactate into the homogenates to test for three times.

2.7. Validation study

Precision and accuracy for the intra- and interday assay of D-lactate in 20 μL of homogenates were performed as follows: 20 μL of homogenate was added with 10 μL of 1.0 mM propionic acid

(I.S.) and 170 μL of lithium D-lactate in CH_3CN was added to a final concentration of 0, 0.25, 0.50 and 1.00 mM, with the same procedure described above thereafter. The precision was calculated as the coefficient of variation (CV %) of six analyses, and the accuracy was acquired as the percentage of the theoretical concentration to the concentration measured by the HPLC method.

2.8. Determination of methylglyoxal (MG)

The determination of methylglyoxal in rat kidney was performed according to previous reports with modifications [29–31]. Briefly, 150 mg of rat kidney was homogenized in 0.5 mL of PBS buffer in a 2-mL screw-cap microcentrifuge tube for 30 s at 6000 rpm with the Precellys 24 homogenizer (Bertin Technologies, France). The homogenate was centrifuged at 12,000 rpm for 15 min at 4 °C, and 320 μL of the supernatant was mixed with 80 μL of 2.25 M perchloric acid (PCA) and allowed to stand for 24 h for deproteinization. After removing the PCA precipitates by centrifuge at 12000 rpm for 10 min at 4 °C, 240 μL of the supernatant was derivatized with 60 μL of 50 mM *o*-PD for 24 h. MG in the sample reacted with *o*-PD to result 2-MQ and the resultant was further centrifuged at 12000 rpm for 10 min at 4 °C for removing the impurities. Finally, 240 μL of the supernatant was added with 48 μL of 5-MQ as internal standard, and 100 μL of aliquot was injected into HPLC. An ODS column (150 mm × 4.6 mm ID., Biotic Chemical. Co., Ltd., Taipei, Taiwan) was used, and the mobile phase was 10 mM NaH_2PO_4/CH_3CN (80/20, v/v) at a flow rate of 1.0 mL/min. UV absorption was monitored at 315 nm for 2-MQ and 5-MQ.

2.9. Histological examination

The left kidney of the rat was removed, decapsulated, and fixed in 10% buffered neutral formalin (pH 7.4) for 24 h, followed by 70%, 85%, 95%, and absolute ethanol for dehydration, and xylene clearing. Then the kidney was embedded in paraffin, sectioned at 3–5 μm in thickness, and stained with periodic acid–Schiff (PAS) stain.

2.10. Statistical analysis

All data were expressed as the mean ± SD. The statistical significance of the difference between groups was analyzed by Student's *t*-test for unpaired data. A *p*-value less than 0.05 was considered significant.

3. Results and discussion

3.1. Body weight, blood glucose and histological findings

The body weight and blood glucose of rats were shown in Fig. 1. The results indicated that the STZ rat was lighter than the control after 1 week of STZ induction (Fig. 1A), and this trend reached statistically significant difference in the second week and thereafter. And the concentration of blood glucose of STZ rat raised above 300 mg/dL after 1 week of administration; while the animal given the vehicle citrate buffer had normal blood glucose levels of below 100 mg/dL (STZ rat versus control; 336.7 ± 67.9 mg/dL vs. 91.0 ± 11.5 mg/dL, respectively, $p < 0.001$) (Fig. 1B). The levels of blood glucose of STZ animals have further risen above 400 mg/dL after the second week and thereafter. The significant difference in the blood glucose levels between the STZ rats and vehicle injected controls indicates that the diabetic stages were successfully induced in the rats.

The treatment-related histopathological changes are presented in Fig. 2. Renal damage was observed only in diabetic rats, characterized by mesangial expansion, glomerular basement membrane uniformly thickened, and tubular hyaline change (Fig. 2B), but not

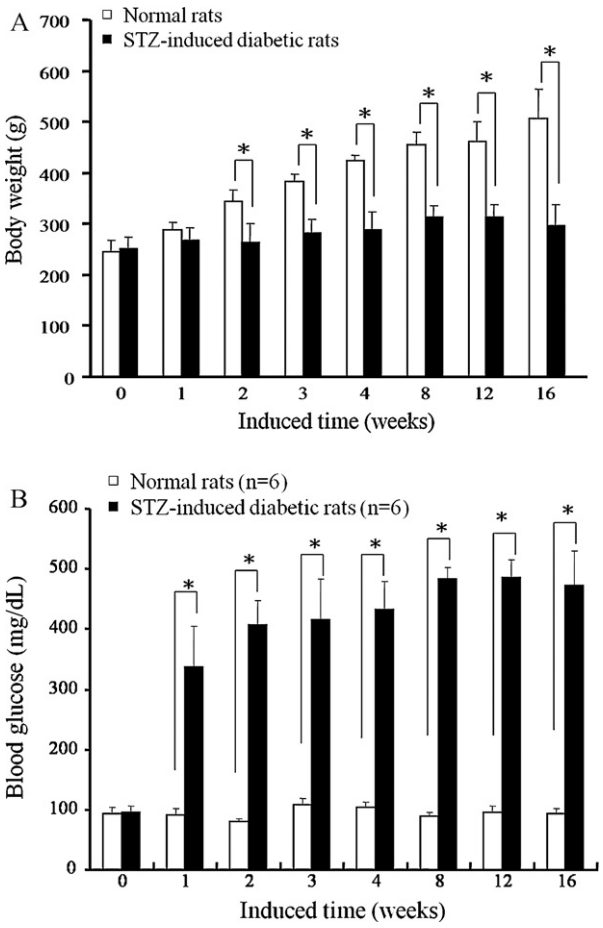

Fig. 1. The body weight (A), and blood glucose (B) of normal (white columns) and STZ-induced (black columns) rats. Results are presented as the mean ± SD ($n = 6$). Significant levels are $^*p < 0.001$ as compared with normal values.

in the normal rats receiving vehicle injections (Fig. 2A). The findings on the histopathological changes in the kidney of STZ rats were in agreement of previous reports. In STZ induced-Lewis rats, the animals developed an increase in mesangial matrix, electron dense material in the mesangium, with immunoglobulin G, C3, and occasionally fibrinogen deposits in the glomerular mesangium in early course of diabetes [32]. Glomerular hypertrophy was observed in the first day after giving 70 mg/kg streptozotocin, and mesangial surface area thickness in the 8th day for SD rats [33]. Shanmugam et al. gave the Wistar rats single dose of STZ to target blood glucose above 250 mg/dL after 3 days. The tubular degeneration, degeneration of glomeruli, focal necrosis of tubules, cystic dilatation of tubules and fatty infiltration were observed in the renal tissues of the animals [34].

3.2. D-Lactate levels in serum, kidney, and liver

The intraday calibration curve for D-lactate was $y = 0.2929X + 0.0962$, showing a good linearity with a correlation coefficient (R^2) of 0.9991. The interday calibration curve was $y = 0.3985X + 0.0411$, with a R^2 of 0.9989. The accuracy values (recovery rate) for D-lactate determination in 20 μL of homogenates were 90.54–117.95%, and the intra- and inter-day precision values were within 13.81 and 13.39%, respectively. The detection limit for D-lactate was approximately 0.5 pmol, which was better than that in our previous report [10]. In the previous study, the D-lactate derivatives was loaded onto an Empore™ SBD-RPS cartridge, and eluted with 100 μL of mobile phase for

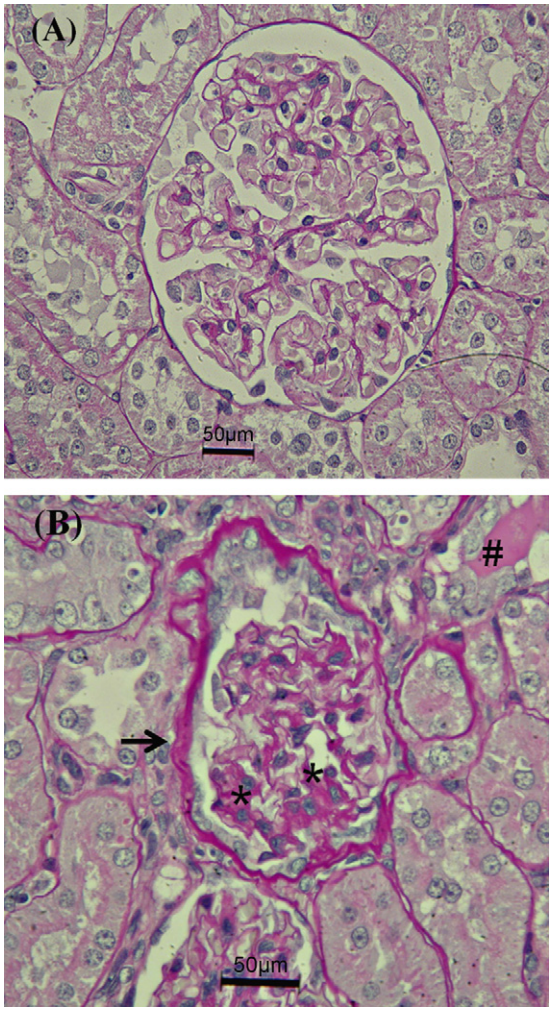

Fig. 2. Light micrograph of normal (A) and diabetic (B) rat renal cortex after 12 weeks of induction. The sections were stained with PAS. The control rats receiving vehicle injections did not exhibit renal morphologic alterations (A). Renal damage was observed in diabetic rats (B), and characterized by mesangial expansion (*), glomerular basement membrane uniformly thickened (arrow), and tubular hyaline change (#) (bar: 50 μm).

elution, whereas in the present study, the volume of elution solution was 200 μL that achieved the more complete elution. In addition, compared with the chromatograms of urine, there are much less peaks in the chromatogram of serum, liver, and kidney homogenates. This may due to the more complicated compositions of organic carboxylic acids in the urine.

The representative chromatograms of the total, D- and L-lactate were shown in Fig. 3, using the samples from the kidney homogenates. Fig. 3A and C, respectively, demonstrated the chromatograms of total (D + L)-lactate for normal and diabetic rats, with the lactates eluted at about 24 min, and the internal standard at about 50 min by an ODS column. The D- and L-lactate were further separated by an AD-RH chiral column, and eluted at about 15 min and 19 min, respectively (Fig. 3B and D).

After STZ treatment, serum D-lactate levels in diabetic rat was significantly elevated from the 4th to 16th week (Fig. 4), and statistically higher than that for normal rats after 4, 8, 12, and 16 weeks of STZ treatment (4.33 ± 1.14 versus 0.99 ± 0.52, 18.07 ± 1.91 versus 2.17 ± 1.90, 18.78 ± 10.01 versus 4.8 ± 3.87, and 22.45 ± 12.14 versus 5.64 ± 4.9 mmol/mg protein, respectively, $p < 0.001$). The D-lactate levels in diabetic rats were about 4, 8, 4, and 4 times as height as that in normal rats after 4, 8, 12, and 16 weeks of treatment, respectively.

The D-lactate levels in kidney and liver also increased significantly in the diabetic rats (Fig. 4). The increase of renal D-lactate level was time-dependent. After 4 weeks of treatment, the D-lactate level of diabetic rats was about 4.8-fold higher than normal rats (2.99 ± 1.45 versus 0.62 ± 0.35 mmol/mg protein, $p < 0.001$). And the D-lactate level was up to 13.11 ± 5.44 mmol/mg protein, and approximately 12.25-fold higher than normal rats in the 8th week (1.07 ± 0.77 mmol/mg protein, $p < 0.001$). At the 12th and 16th weeks, the D-lactate level increased to more than 27-fold higher than that in the normal rats (18.19 ± 1.86 versus 0.68 ± 0.55 for 12th week, and 23.23 ± 12.98 versus 0.82 ± 0.23 mmol/mg protein for 16th week, respectively. Both $p < 0.001$). Similar increases were observed for the liver homogenates. The hepatic D-lactate levels in diabetic rats were about 3, 6, 10, and 5 times as high as that in normal rats after 4, 8, 12, and 16 weeks of treatment, respectively (diabetic rat versus control; 3.66 ± 1.46 versus 1.38 ± 0.84, 6.05 ± 0.94 versus 1.08 ± 0.48, 9.29 ± 1.69 versus 0.9 ± 1.9, and 9.95 ± 5.5 versus 1.85 ± 0.59 mmol/mg protein, for 4, 8, 12, and 16 weeks, respectively, all tests were $p < 0.001$). These results showed that the renal and hepatic D-lactate levels in rats were significantly increased after STZ induction.

However, the patterns of the alteration were different in the serum, liver and kidney. The serum D-lactate levels of diabetic rats did not increase continuously after STZ induction. The ratio of serum D-lactate levels between diabetic and normal rats was highest after 8 weeks of induction, but declined after 12 and 16 weeks. The hepatic D-lactate levels were stable after 16 weeks of treatment, with the highest ratio for diabetic and normal rats at the 12th week. Only renal D-lactate level continuously increased in the progress of diabetes and might be closer to correlate with the progress of diabetics.

The second interesting finding was that the D-lactate in kidney became much higher than those in liver of STZ rats, suggesting possible difference on the tissues' abilities to synthesize D-lactate. Glyoxalases, the key enzymes to metabolize MG to D-lactate, is composed of two enzymes: glyoxalase I, which metabolizes MG to *S*-D-lactoylglutathione, and glyoxalase II, which converts *S*-D-lactoylglutathione to D-lactate [35]. These enzymes were found to explicit different activities in liver and kidney in diabetic rats [36]. After using alloxan to induce diabetes in rats, the activity of glyoxalase I was increased in kidney, but decreased in liver [36]. Study on the effect of the aldose reductase inhibitor, statil, also found the activities of glyoxalase I and II were decreased in the liver of the STZ treated diabetic rats, but the concentrations of MG in the kidney cortex and medulla were increased in diabetic rats, comparing to that in the controls [37]. Thus the higher D-lactate levels in kidney than those in liver showed in current study might be due to the different activities of glyoxalases in kidney and liver.

3.3. Elevation of methylglyoxal in diabetic rat kidney

We further determined renal MG levels by HPLC in rats, and found that it was about 1.25-fold higher than normal rats at the 12th week ($p < 0.01$) (Fig. 5). MG appears to be associated with diabetic complications through reacting with proteins to form advanced glycation end products (AGEs), actioning on energy production, inducing free radical generation and finally causing the death of cells [38]. D-Lactate in animal physiologic fluids could be originated from digestion or bacterial production in the intestinal tract, or from endogenous production by the methylglyoxylase pathway [38,39]. The concurrent increase of renal MG level provided the evidence of that the underlying mechanism of D-lactate elevation in serum, liver and kidney of diabetic rats was resulted from MG production.

It was interesting to find that the serum D-lactate concentration in the normal rats was apparently increased in this study. As

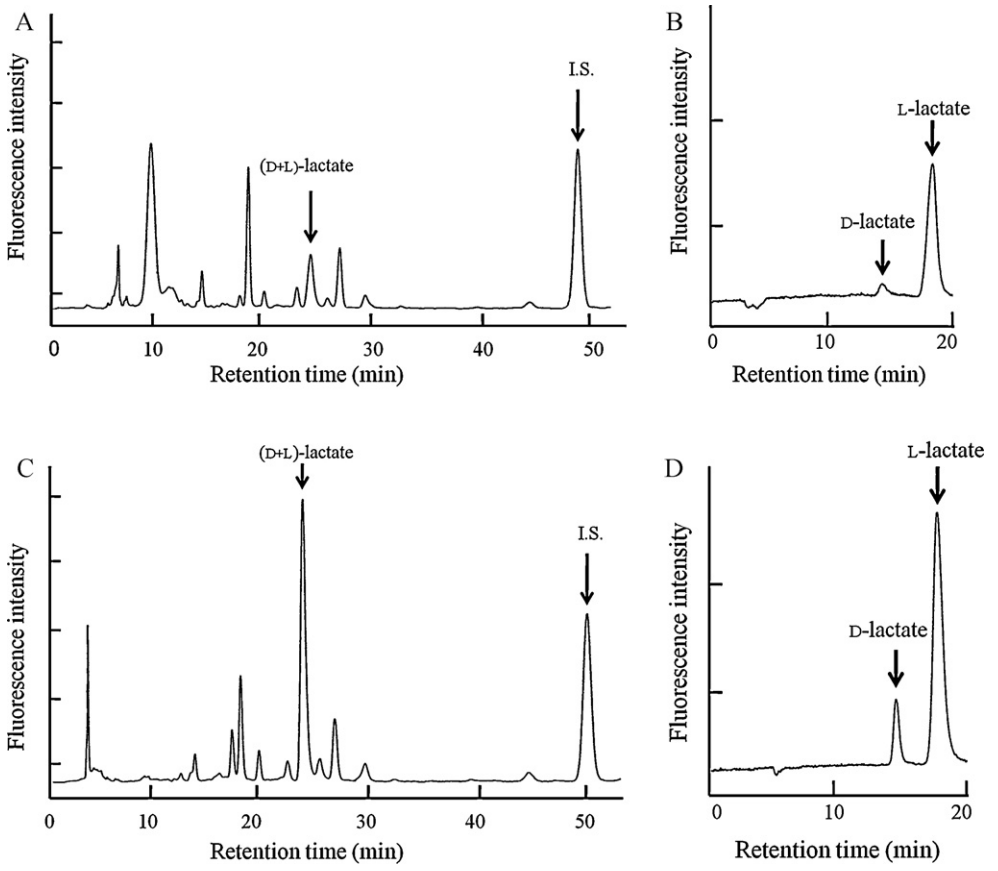

Fig. 3. HPLC chromatograms of enantiomeric analysis of D,L-lactate in normal and diabetic kidney homogenates. Total lactate was first separated from normal (A) and diabetic (C) kidney homogenates, and lactate was further separated into D- and L-lactate as shown in (B) and (D) for normal and diabetic kidney homogenates, respectively. In diabetic rat kidney homogenates, significant elevation of D-lactate was found. I.S., internal standard (propionic acid).

a physiological metabolite formed in vivo by the fragmentation of triose-phosphates and the catabolism of ketone bodies and threonine, MG reacts with arginine and lysine residues in protein to form AGEs [40]. AGEs accumulated in many aging-related chronic diseases, such as atherosclerosis, diabetes, arthritis and neurodegenerative disease [40,41]. The increase of AGEs in lens proteins and skin collagen was correlated with aging, and the accumulation of AGEs was detectable in these tissue proteins in diabetes [40]. The apparent increase of serum D-lactate concentration in the normal rats in Fig. 4 may be related to aging.

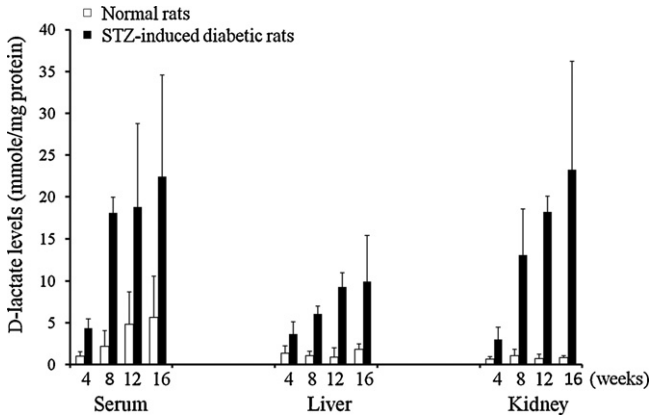

Fig. 4. D-Lactate concentrations in serum, liver, and kidney of normal and diabetic rats after 4, 8, 12, and 16 weeks of vehicle or STZ treatment. Results are presented as the mean ± SD ($n = 6$). Significant levels are $^{*}p < 0.001$ as compared with control values.

The renal histopathological changes were only observed in the diabetic rats with the increased D-lactate level in previous and our reports [10]. Additionally, we found that renal D-lactate and MG concentrations raised concurrently. These results together provide the clues that increased renal MG level in diabetic rats may be associated with diabetic nephropathy, and the changes of D-lactate might be able to early reflect the process of nephropathy.

In conclusion, the concentrations of D-lactate in the tissues of normal and diabetic rats after 4, 8, 12 and 16 weeks of administration were determined by column-switching HPLC with fluorescence detection. The results showed that D-lactate levels in the kidney of diabetic rats increased continuously after STZ induction, and with the histological damage of renal tissue. These results

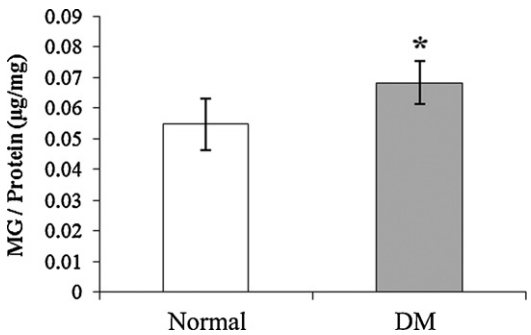

Fig. 5. Concentrations of MG in the kidney homogenates of normal and diabetic (DM) rats after 12 weeks of vehicle or STZ treatment. Results are presented as the mean ± SD ($n = 4$). $^{*}p < 0.01$ vs normal rats.

suggested that concentration and percentage of D-lactate in the kidney may be related with the renal damage in diabetics.

Acknowledgments

The authors express their sincere appreciation to Prof. K. Imai (Musashino University, Tokyo, Japan), and Prof. T. Fukushima (Toho University, Chiba, Japan) for their helpful suggestions. This work was financially supported by the National Science Council of the Republic of China (NSC92-2320-B-038-041).

References

[1] S.M. Smith, R.H.K. Eng, F. Buccini, J. Infect. Dis. 154 (1986) 658.
[2] S.M. Smith, R.H. Eng, J.M. Campos, H. Chmel, J. Clin. Microbiol. 27 (March (3)) (1989) 385.
[3] M.J. Murray, J.J. Barbose, C.F. Cobb, J. Surg. Res. 54 (1993) 507.
[4] M.J. Murray, M.D. Gonze, L.R. Nowak, C.F. Cobb, Am. J. Surg. 167 (1994) 575.
[5] E. Günel, O. Çağlayan, F. Çağlayan, Pediatr. Surg. Int. 14 (1998) 59.
[6] M. Poeze, A.H.M. Froon, J.W.M. Greve, G. Ramsay, Br. J. Surg. 85 (1998) 1221.
[7] F. Cağlayan, M. Cakmak, O. Cağlayan, T. Cavuşoglu, J. Invest. Surg. 16 (2003) 233.
[8] M. Demircan, F. Caglayan, M. Deysine, J.J. Fath, J. Invest. Surg. 17 (2004) 173.
[9] T. Fukushima, J.A. Lee, T. Korenaga, H. Ichihara, M. Kato, K. Imai, Biomed. Chromatogr. 15 (2001) 189.
[10] J.A. Lee, Y.C. Tsai, H.Y. Chen, C.C. Wang, S.M. Chen, T. Fukushima, K. Imai, Anal. Chim. Acta 534 (2005) 185.
[11] H. Hasegawa, T. Fukushima, J.A. Lee, K. Tsukamoto, K. Moriya, Y. Ono, K. Imai, Anal. Bioanal. Chem. 377 (2003) 886.
[12] P.J. Beisswenger, S.K. Howell, A.D. Touchette, S. Lal, B.S. Szwergold, Diabetes 48 (1999) 198.
[13] P.J. Thornalley, N.I. Hooper, P.E. Jennings, C.M. Florkowski, A.F. Jones, J. Lunec, A.H. Barnett, Diabetes Res. Clin. Pract. 7 (1989) 115.
[14] M.M. Christopher, J.D. Broussard, C.W. Fallin, N.J. Drost, M.E. Peterson, Metabolism 44 (1995) 287.
[15] Y. Kondoh, M. Kawase, Y. Kawakami, S. Ohmori, Res. Exp. Med. (Berl.) 192 (1992) 407.
[16] Y. Kondoh, M. Kawase, M. Hirata, S. Ohmori, J. Biochem. 115 (1994) 590.
[17] A.C. McLellan, P.J. Thornalley, J. Benn, P.H. Sonksen, Clin. Sci. 87 (1994) 21.
[18] R.B. Brandt, S.A. Siegel, M.G. Waters, M.H. Bloch, Anal. Biochem. 102 (1980) 39.
[19] S. Girotti, S. Ghini, G. Carrea, R. Bovara, A. Roda, R. Budini, Anal. Chim. Acta 255 (1991) 259.
[20] T. Fukushima, S. Adachi, H. Ichihara, S. Al-Kindy, K. Imai, Biomed. Chromatogr. 13 (1999) 418.
[21] D. Mathis, L. Vence, C. Benoist, Nature 414 (2001) 792.
[22] K.R. Mansford, L. Opie, Lancet 1 (1968) 670.
[23] C.C. Rerup, Pharmacol. Rev. 22 (1970) 485.
[24] W.J. Schnedl, S. Ferber, J.H. Johnson, C.B. Newgard, Diabetes 43 (1994) 1326.
[25] National Diabetes Data Group, Diabetes 28 (1979) 1039.
[26] M.M. Bradford, Anal. Biochem. 72 (1976) 248.
[27] Y.C. Tsai, T.H. Liao, J.A. Lee, Anal. Biochem. 319 (2003) 34.
[28] Y.C. Tsai, Y.C. Chou, A.B. Wu, C.M. Hu, C.Y. Chen, F.A. Chen, J.A. Lee, Life Sci. 78 (2006) 1385.
[29] S. Ohmori, M. Mori, M. Kawase, S. Tsuboi, J. Chromatogr. – Biomed. Appl. 414 (1987) 149.
[30] F.W.R. Chaplen, W.E. Fahl, D.C. Cameron, Anal. Biochem. 238 (1996) 171.
[31] A. Dhar, K. Desai, J. Liu, L. Wu, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 877 (2009) 1093.
[32] R. Weil, M. Nozawa, M. Koss, C. Weber, K. Reemtsma, R.M. McIntosh, Surgery 78 (1975) 142.
[33] C. Sassy-Prigent, D. Heudes, C. Mandet, M.F. Bélair, O. Michel, B. Perdereau, J. Bariéty, P. Bruneval, Diabetes 49 (2000) 466.
[34] K.R. Shanmugam, K. Mallikarjuna, K. Nishanth, C.H. Kuo, K.S. Reddy, Food Chem. 124 (2011) 1436.
[35] P.J. Thornalley, Biochem. J. 269 (1990) 1.
[36] N. Baquer, D. Gupta, J. Raju, Indian J. Clin. Biochem. 13 (1998) 63.
[37] S.A. Phillips, D. Mirrlees, P.J. Thornalley, Biochem. Pharmacol. 46 (1993) 805.
[38] P.J. Thornalley, Gen. Pharmacol. 27 (1996) 565.
[39] P.J. Thornalley, Mol. Aspects Med. 14 (1993) 287.
[40] T.P. Degenhardt, S.R. Thorpe, J.W. Baynes, Cellular and Molecular Biology, 44, Noisy-le-Grand, France, 1998, p. 1139.
[41] J.W. Baynes, Exp. Gerontol. 36 (2001) 1527.