日本財団 助成事業
The Nippon Foundation

２００１年度日中医学協会共同研究等助成事業報告書

－在留中国人研究者研究助成－

2002 年 2 月 7 日

財団法人 日中医学協会
理事長 殿

研究者氏名 李 冰 ㊞
所属機関名 新潟大学医学部附属腎研究施設機能制御分野
指導責任者氏名 遠手 巍
職名 教授
所在地 〒951-8510 新潟市旭町通1-757
電話 025-227-2158 内線 2158

1．研究テーマ

新しい糸球体及び腎間質の血行動態測定法・糖尿病モデルの検討

2．本年度の研究業績

(1) 学会・研究会等における発表 (有) ・ 無 （学会名・演題）

第34回米国腎臓学会，San Franciso, 2001,10.
Real-time observation of renal hemodynamic changes in diabetic rats
Bing Li, Tetsuo Morioka, Jian Yao, Takashi Oite
第34回米国腎臓学会，San Franciso, 2001,10.
Corordination of mesangial cell contraction by gap junction -mediated intercellular Ca wave
Jian Yao, Tetsuo Morioka, **Bing Li**, Takashi Oite
第44回日本腎臓学会学術総会，東京，2001,5.
高血糖状態における培養ヒト糸球体内皮細胞のＮＯ産生能
Mari hoshiyama, **Bing Li**, Tetsuo Morioka, Jian Yao, Takashi Oite
第44回日本腎臓学会学術総会，東京，2001,5.
Endothelin is a potent inhibitor of MMP-2 secretion and activation in rat mesangial cells
Jian Yao, Tetsuo Morioka, **Bing Li**, Takashi Oite

(2) 学会誌等に発表した論文 (有) ・ 無 （雑誌名・論文名）

Bing Li, Jian Yao, Tetsuo Morioka, Takashi Oite
Nitric oxide increases albumin permeability of isolated rat glomeruli via a phosphorylation-dependent mechanism
Journal of the American society nephrology 12: 2616-2624, 2001

Jian Yao, Tetsuo Morioka, **Bing Li**, Takashi Oite
Endothelin is a potent inhibitor of MMP-2 secretion and activation in rat mesangial cells
Am J Physiol Renal Physiol 280: F628-F635, 2001

3．今後の研究計画

1．前期にはStreptozotocin 投与によるラット糖尿病モデルを用いて、その糸球体微小循環系動態をリアルタイムで画像解析してきた。その結果、経過とともに糸球体輸入・輸出動脈の拡大、血流速度の増大が確認された。後期は、その成因を糸球体輸入・輸出動脈の血管作動性物質に対する反応性の変化にあると仮定して研究を進める。

2．血管収縮作用を持つアンギオテンシン II, エンドセリンを投与して、糖尿病初期、完成期における糸球体輸入・輸出動脈の直径、同部位の血流速度をリアルタイム測定して、収縮反応に対する糖尿病の影響を解析する。

3．血管弛緩作用を持つ一酸化窒素ドナーを投与して、２と同様の時期のラットで、同様の検索を行う。

4．アンギオテンシン II 受容体阻害剤、エンドセリン受容体阻害剤、一酸化窒素合成阻害剤の短期多量投与により、２，３の影響の各血管作動性物質特異性について検討する。

5．４の薬剤とアンギオテンシン転換酵素阻害剤を継続投与し、糖尿病における糸球体微小循環系変化に対する治療効果を検索する。

6．上述の実験結果を元に、糖尿病性腎症の発症機構に腎糸球体での微小循環動態の変化が大きく関与しているか否かを検証し、その結果を原著論文にまとめる。

4．指導責任者の意見

李氏は、微小血管血行動態実時間観察のために私どもの教室で開発した、共焦点レザー顕微鏡・高速冷却 CCD ビデオ撮影装置（Y. Oyanagi-Tanaka et.al. *Kidney International* 59:252, 2001）を用いて、この半年間、精力的な研究を行い、成果をあげてきた（研究業績の項、参照)。特に貴財団の支援によった研究テーマは昨年末の米国腎臓学会（サンフランシスコ）で発表され、注目された。現在、上述の研究計画に従い研究を進め、原著論文にまとめるための作業を進めている。

李氏の研究意欲、業績に関しては申し分無く、是非とも貴財団の研究助成の継続をお願いし、最終的な原著論文作成の実現に対してご支援をお願いしたい。

李氏は、新潟大学医学部腎研究施設での先端医学研究プロジェクトの一員として研究・研修のキャリアを積んできた。大学院博士課程終了後は、国際的な腎臓学者を目指してさらに研究・研修を続け、将来の中国・日本の医学・医療の発展に貢献しうる人材と信じている。

指導責任者氏名 追手 巍 ㊞

5．研 究 報 告 書

別紙報告書作成要領により、添付の用紙で研究報告書を作成して下さい。

研究発表中または研究中の本人のスナップ写真を添付して下さい。

※研究成果を発表する場合は、発表原稿・抄録集等も添付して下さい。

※発表に当っては、*日中医学協会助成金による*旨を明記して下さい。

—日中医学協会助成事業—

新しい糸球体及び腎間質の血行動態測定法・糖尿病モデルの検討

研究者氏名　李　氷
中国所属機関　ハルビン医科大学第二附属病院小児科
日本所属機関　新潟大学医学部附属腎研究施設機能制御学分野
指導責任者　教授　追手　巍
共同研究者名　姚　建，森岡　哲夫

要　旨：

Diabetic nephropathy is the leading cause of end-stage renal diseases. The progression of diabetic nephropathy is closely related to disturbance of glomerular hemodynamics, such as renal hyperperfusion and/or glomerular hyperfiltration. Therefore, the aim of this study is to observe and analyze the alteration of renal he modynamics in diabetic rats in vivo using confocal laser scan microscope (CLSM). Experiments were performed in Munich-Wistar rats on days 4 and 28 after streptozotocin (STZ) injection. Blood and urinary glucose levels, urinary protein excretion and creatinin clearance were estimated. Microalbuminuria level was also examined by radioimmunoassay. A polyethylene catheter was inserted into the carotid artery to allow blood pressure measurement. The left kidney weight (KW) was also estimated. We measured the glomerular size using the isolated glomeruli from control and diabetic groups. On days 4 and 28 after STZ injection, we examined hemodynamic changes by an intravital microscope equipped with real-time CLSM in combination with a high-speed CCD video camera. To measure vessel diameter and erythrocyte velocity, rats were injected with fluorescein isothiocyanate (FITC)-labeled dextran and FITC-labeled red blood cells (RBCs). The diabetic rats on days 4 and 28 after STZ injection had a significantly higher ratio of kidney/body weight than control rats. Also, glomerular size in diabetic rats was significantly larger than that in control groups. There was not significant difference in mean arterial pressure (MAP) between diabetic and control rats. On days 4 and 28, th e diameters of afferent arterioles (AA) and efferent arterioles (EA) significantly increased in diabetic rats as compared with control rats. Moreover, erythrocyte velocities within glomeruli appeared to be faster in diabetic rats than in control rats. In a ddition, glomerular blood flow (GBF) was significantly higher in diabetic rats on days 4 and 28 after STZ injection as compared with control rats. Our real-time observations demonstrated that renal hypertrophy and glomerular hyperperfusion had started as e arly as at 4 days of diabetes. The noninvasive procedure, using CLSM in combination with high-speed video camera, allowed us to evaluate the glomerular microcirculation in diabetic condition in vivo, and to reconfirm the significance of hemodynamic changes in diabetic nephropathy.

Key words: CLSM, diabetes, glomerular hemodynamics, AA, EA

緒　言

Diabetic nephropathy is the leading cause of end-stage renal disease. Introrenal hemodynamic alterations, as manifest by glomerular hyperfiltration and hyperperfusion, are thought to be the foremost factors responsible for the onset and progression of diabetic nephropathy. Experimentally, glomerular hyperfiltration has been shown to result from elevations in the glomerular capillary blood flow and the glomerular capillary hydraulic pressure. Glomerular circulation is mainly regulated by two resistance arterioles, the afferent arteriole and efferent arteriole. The afferent and efferent arterioles normally constrict and dilate in response to changes in systemic blood pressure to maintain

glomerular filtration while protecting the glomerulus from excessive pressure. Therefore, real-time observation of glomerular hemodynamic changes would allow us to deepen our understanding of progressive mechanisms in diabetic nephropathy.

Recently, glomerular function has been directly evaluated with the use of the isolated perfused glomerulus, juxtamedullary nephrons, hydronephrotic kidneys and needle-probe charge-coupled device (CCD) videomicroscope. These approaches, however, invasive manipulations that might alter the renal vascular responsiveness, and may thus confound the characterization of these renal vessels. We have developed a new technique to directly visualize the glomerular microcirculation with the CLSM in combination with a high -speed CCD video camera. The particular advantage of this system over other methods is to avoid nonphysiological effects of invasive operative procedures, and permit the direct evaluation of renal microcirculation in vivo under physiological and pathological conditions. In this present study, we directly observed the alteration of hemodynamics in diabetic rats using our newly developed videomicroscopic technique.

対象と方法

Animals

Studies were performed in male Munich Wistar rats of six- to seven-week-old, which purchased from Simonsen Laboratories, Inc. Diabetes were induced by intraperitoneal injection of streptozotocin (sigma) (65mg/kg body wt) dissolved in 0.1M sodium citrate buffer, PH 4.5. Diabetic and control rats were followed for 4 and 28 days. All rats received standard chow and tap water.

Intravital observation of the renal microcirculation

Observations of microcirculation blood flow were made with an intravital microscope system (Nikon, Tokyo, Japan), equipped with a real-time confocal scanner unit model CSU10 and image processing devices. Differing from conventional CLSM, the real-time CLSM system used in this study CSU10 (YOKOGAWA Electric Corporation, Tokyo, Japan) has two disks: microlens array and pinhole array. The CSU10 unit is designed to attain a high signal to noize ratio by minimizing the background light inside the scanner, thus making observation of weak fluorescent specimens possible. Rotation speed of the scanner motor is 1800 rpm. The scanner actually captures 360 frames of confocal images which one can observe at the eyepiece of the scanner, and sends 30 frames of confocal images per second to an ICCD camera (Model C2400-89; HAMAMATSU PHOTONICS K.K., Shizuoka, Japan), to synchronize with the scanning rate of the camera and be video-recorded (Model SVO-9600; Sony, Tokyo, Japan) for later analysis by using of a recording lens and salt water immersion objectives.

The Munich Wistar rats were anesthetized by intraperitoneal injection of thiobutabarbital sodium salt (100mg/kg). The body temperature of animals was kept 37.0 C on a heating pad. Polyethylene catheters (PE50) were inserted into the carotid artery for blood pressure measurements and into the femoral vein for administrating labeled plasma component and autologous red blood cells (RBCs). The left kidney was exposed by a flank incision and split longitudinally. To analyze the microcirculation from the surface of kidney, the kidney was placed under the CLSM. The kidney was immersed in a bath of physiological saline (Na^+ 154mEq/L, Cl^- 154mEq/L) in which the temperature was kept at 37±1 C.

Measurement of microhemodynamic parameters in glomerular tufts

Measurement of vessel diameter. To measure vessel diameter, enhancement of the contrast of microvessel images against a dark background was made by intravenous injection (10 mg ml^{-1}, 2 ml kg^{-1}) of a solution of FITC-labeled dextran (FITC-Dx, MW 150,000; Sigma Chemical Co., St. Louis, MO). This fluorescent staining procedure produced bright fluorescent images of the vascular lumen, and enabled adequate mapping of the luminal diameter. Diameters of microvessels were measured with a vernier caliper on individual frames of the video-recorded images.

Erythrocyte Velocity. To measure erythrocyte velocity, a batch of erythrocytes labeled with FITC was injected intravenously. Briefly, washed erythrocytes obtained from an experimental rat were incubated with a PBS (137mM NaCl, 6.4mM Na_2HPO_4, 2.7mM KCl, 1.5mM KH_2PO_4, pH 7.8) solution containing 1mg/ml FITC (ICN Pharmaceutical, Inc., Cleveland, OH, USA). The labeled cells were then washed twice with a saline solution containing 1% bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, MO) to remove unconjugated fluorescent dye. The final volume percent of the labeled cells was adjusted approximately to 50% by adding an isotonic saline solution, and an aliquot of these suspensions was injected (1ml/Kg) through the tail vein of the rat to measure centerline erythrocyte velocity. The rate of the labeled RBC in total RBC was about 1% by a single calculation. From the video-recorded images, erythrocyte velocity was calculated by frame-by-frame analysis, more than five different areas were measured, and averaged for at least five measurements.

Measurement of Glomerular Volume

After measurement of kidney weight, the outer 1 to 2 mm of the renal cortex in left kidney was excised and cut into fine fragments. The glomeruli were then isolated in DMEM by standard sieving techniques as described previously. The PH of the medium was adjusted to 7.4 before use. The whole procedure for isolation of glomeruli was carried out within 15 minutes at room temperature. Isolated glomeruli were allowed to adhere to an observation chamber coated with poly-L-lysine (1 mg/ml) for 5 to 10 seconds. Unattached glomeruli were removed by gentle washing with fresh isolation medium. Adherent glomeruli were viewed in a microscope, and then the image was captured by computer. The area of each glomerulus was automatically measured using NIH software. The volume of each glomerulus was calculated from the area (S) using the formula $V = 4/3\pi(S/\pi)$. At least 120 glomeruli from three or more rats were studied in each experiment.

Statistical analysis

All measured values are given as mean ± standard deviation (SD). The nonparametric Mann -Whitney U test was used for comparison of the measured values of erythrocyte velocities, since these parameters are known to have a distribution which deviates considerably from the Gaussian. A P value less than 0.05 was considered significant in all statistical tests.

結 果

Determination of baseline characteristics of experimental animals

At 4 and 28 days after STZ injection, determination of baseline characteristics differed significantly between the investigated groups as showed in Table 1. Blood and urinary glucose levels, urinary protein excretion and creatin in clearance were estimated. Microalbuminuria level was also examined by radioimmunoassay. Blood glucose, urine glucose and albuminuria were markedly elevated in diabetes animals. Hyperglycemia (> 300 mg/dl) has already started at day 4, and lasted at day 28. Heavy proteinuria (>100 mg/day) started at day 4, and reached at maximum (mean = 500 mg/day) at day 28. Mean arterial pressure of the carotid artery in the control rats was 107±3mmHg. On days 4 and 28 after injection with STZ, mean arterial pressure was 106±4 mmHg and 101±9mmHg, respectively, not significant difference with control.

Kidney weight and ratio of kidney/ body weight

Although body weight was less in diabetic rats after 4 and 28 days injection, diabetic kidneys weighed more than control kidneys. The ratio of Kidney/body weight in 4-day diabetic rats was significantly higher than that in control rats, and it was more pronounced in 28-day diabetic group.

The effect of diabetes on erythrocyte velocity

The left kidneys of Munich Wistar rats were observed by intravital microscopy. On day 4 after STZ injection, erythrocyte velocities within glomeruli were slightly faster in diabetic rats (676.1 ± 209.6 μm/sec) than in control

rats (496.1 ± 134.1 μm/sec), similarly, erythrocyte velocities within microvasculature around Bowman's capsule were also slightly faster in diabetic rats (446.1 ± 223.8 μm/sec) than in control rats (238.8 ± 122.4 μm/sec). On day 28 after STZ injection, erythrocyte velocities within glomeruli were significantly faster in diabetic rats (859.3 ± 192.1 μm/sec) than in control rats (495.4 ± 110.7 μm/sec). Therefore, erythrocyte velocities within glomeruli appeared to be faster in diabetic rats than in control rats as early as on day 4. Similarly, on day 28 erythrocyte velocities within microvasculature around Bowman's capsule were also markedly faster in diabetic rats (516.3 ± 114.0 μm/sec) than in control rats (351.3 ± 91.6 μm/sec).

The effect of diabetes on diameters of AA and EA

On days 4 and 28 after STZ injection, we measured the diameters of AA and EA in control and diabetic rats. The afferent and / or efferent arterioles within the glomerui were identified by observation of the microvascular branches and the direction of movement of labeled red blood cells. On day 4 after STZ injection, the diameters of AA in diabetic rats (10.39 ± 1.43 μm) significantly increased, comparision with control rats (8.43 ± 0.52 μm). In addition, the diameters of EA in diabetic rats (10.43 ± 1.70 μm) significantly increased as compared with control rats (8.00 ± 0.68 μm). On day 28 after STZ injection, the diameters of AA in diabetic rats (11.68 ± 0.79 μm) further enlarged, significantly greater increased than that in controls (8.88± 1.22 μm). However, the diameters of EA in diabetic rats (10.49 ± 1.16 μm) did not further increased, but there was significant difference with controls (8.33 ± 0.96 μm).

Measurement of glomerular volume

Using isolated glomeruli from diabetic and control groups, we observed directly glomerular size and calculated the volume of glomeruli. We demonstrated that the volume of glomeruli were markedly larger in diabetic rats as compared with control rats.

The effect of diabetes on glomerular blood flow (GBF)

In order to deepen understanding the importance of hemodynamic alterati on in diabetes, we calculated the change of blood flow using the equation $BF = \pi (D/2)^2 * GBV$. GBF were significantly higher in 4-day diabetic rats as compared with control rats, and it more pronounced in 28 -day diabetic group.

考 察

In the present study, we evaluated the glomerular microcirculation in diabetic rats by intravital microscope equipped with real-time CLSM in combination with a high -speed CCD video camera. This system visualizes AA, EA, and the movement of labeled-RBCs within glomeruli and microvessels around Bowman's capsule. Therefore, GBV, BBV and GBF can be calculated. This research demonstrated that a basal vosodilation of afferent arterioles and efferent arterioles and an increased glomerular blood flow in diabetic rats, consistent with pr evious studies. This phenomenon appeared as early as on day 4 after STZ injection. It deepened our understanding on hyperperfusion and hyperfiltration in early phase of diabetes, and to reconfirm the significance of hemodynamic abnormities in diabetic nephropathy.

The CLSM intravital videomicroscopic technique appears to exceed other methodological approaches in following several points: (1) This technique avoids nonphysiological effects of invasive operative procedures. (2) This system can real-time observe and record the continuous images of movement of FITC-labeled RBCs and calculate the erythrocyte velocities within glomeruli and microvessels around Bowman's capsule, because this system can capture 360 frames of confocal images, and sends 30 frames of confocal images per second to an ICCD camera (3) The diameters of afferent arterioles, efferent arterioles and glomerular blood flow can be evaluated. (4) Systemic hemodynamics (e.g., blood pressure) can be measured simultaneously. (5) The technique and experimental preparation are simpler than other approaches of direct visualization of the renal microcirculation.

Using this newly developed videomicroscopic technique, we evaluated the glomerular microcirculation in normal and diabetes in Munich Wistar rats. The unique characteristic of this rat strain is that many glomeruli are located near the surface of the kidney and easy to visualizing the renal microcirculation by intravital microscopy. We have observed differences of renal hemodynamics between two groups in several points. Firstly, the diabetic rats on days 4 and 28 after STZ injection had a significantly higher ratio of kidney/ body weight than control rats. Also, glomerular volume predominantly enlarged in diabetes as compared with controls. Secondly, on days 4 and 28, the diameters of afferent and efferent arterioles significantly increased in diabetic rats as compared with control rats. Thirdly, on day 28, erythrocyte velocities within glomeruli and vessels around Bowman's capsule were significantly faster in diabetic rats than in control rats. Moreover, on day 4, erythrocyte velocities within glomeruli were significantly faster in diabetic rats than in control rats. Finally, GBF was significantly higher in diabetic rats on days 4 and 28 after STZ injection as compared with control rats.

Although body weight was less in the diabetic rats, diabetic kidneys weighed 22% more than control kidneys in 4-day group. There is a higher ratio of kidney/ body weight in 4-day groups (diabetes 6.00 $\pm$ 0.55, control 4.43 $\pm$ 0.36) and more pronounced in 28-day groups (diabetes 7.36 $\pm$ 0.73, control 4.27 $\pm$ 0.50). Moreover, we also directly observed that glomerular volume significantly increased in diabetes as compared with control rats. While kidney weight was a strong predictor of glomerular filtration rate as previous report. Glomerular hyperfiltration in early diabetes correlates with increased kidney size. On the other hand, we demonstrated that GBV and GBF in diabetic rats of 4 and 28 days had significantly increased as compared with control rats. Therefore, our evidence suggested that diabetes of 4 day had appeared not only renal hypertrophy, but also hyperperfusion and hyperfiltration simultaneously.

The glomerular microcirculation regulates glomerular filtration and renal hemodynamics by altering the vascular resistance of afferent arterioles and efferent arterioles. Therefore, their behavior is the most important determinant of glomerular blood flow and the glomerular filtration rate. In this present study, we observed that the diameters of afferent and efferent arterioles significantly increased in diabetic rats of 4 and 28 days as compared with control rats. Actually, basal diameters of all vessel types were wider in diabetic rats than that in control rats. Diabetic rats showed a reduced renal vascular resistance, which may be caused by increased NO production in the renal microcirculation. We demonstrated that the EA also dilated in the early phase of diabetes besides of AA. However, the dilation capacity of EA was lower than AA. Therefore, the diameters of AA further enlarged with time, while the diameters of EA on day 28 had no difference with that on day 4. In addition, we demonstrated that the AA/EA ratio increased in diabetes of 28 days, indicating a pronounced increased internal glomerular pressure.

This newly developed technique can analyze acute changes induced by some factors in glomerular microcirculation in vivo. To shed some light on this area, the effects of some vasodilator and vasoconstrictor on renal microcirculation should be investigated under direct intravital microscopic observation. That is our important forthcoming work.

The noninvasive procedure, using CLSM in combination with high-speed videocamera, allowed us to evaluate the renal microcirculation in vivo. It is useful to analyze directly mediators of diabetic hyperfiltration and other experimental models. This technique for directly studying afferent and efferent arterioles changes and glomerular filtration rate in vivo may provide important in sights into the actions of drugs and into renal diseases. Clinicians are beginning to be able to select drugs that have desired effects on the renal microcirculation.

参考文献

1. Anderson, S., and J.P. Vora. 1995. Current concepts of renal hemodynamics in di abetes. *J Diabetes Complications*. 9:304-7.

2. De Vriese, A.S., M.S. Stoenoiu, M. Elger, O. Devuyst, R. Vanholder, W. Kriz, and N.H. Lameire. 2001. Diabetes-induced microvascular dysfunction in the hydronephrotic kidney: role of nitric oxide. *Kidney Int.* 60:202-10.

3. Hostetter, T.H. 1994. Mechanisms of diabetic nephropathy. *Am J Kidney Dis*. 23:188-92.

4. Hostetter, T.H. 2001. Hypertrophy and hyperfunction of the diabetic kidney. *J Clin Invest*. 107:161-2.

5. Ito, S., and O.A. Carretero. 1990. An in vitro app roach to the study of macula densa-mediated glomerular hemodynamics. *Kidney Int*. 38:1206-10.

6. Loutzenhiser, R., M. Epstein, K. Hayashi, and C. Horton. 1990. Direct visualization of effects of endothelin on the renal microvasculature. *Am J Physiol*. 258:F61-8.

7. McMillan, D.E. 1984. The microcirculation in diabetes. *Microcirc Endothelium Lymphatics*. 1:3-24.

8. Miura, K., and M. Minamiyama. 1998. [In vivo visualization of renal microcirculation using hydronephrotic rat kidney]. *Nippon Yakurigaku Zasshi*. 112:251-6.

9. O'Bryan, G.T., and T.H. Hostetter. 1997. The renal hemodynamic basis of diabetic nephropathy. *Semin Nephrol.* 17:93-100.

10. Ogasawara, Y., K. Takehara, T. Yamamoto, R. Hashimoto, H. Nakamoto, and F. Kajiya. 2000. Quantitative blood velocity mapping in glomerular capillaries by in vivo observation with an intravital videomicroscope. *Methods Inf Med*. 39:175-8.

11. Ohishi, K., and P.K. Carmines. 1995. Superoxide dismutase restores the influence of nitric oxide on renal arterioles in diabetes mellitus. *J Am Soc Nephrol*. 5:1559-66.

12. Oyanagi-Tanaka, Y., J. Yao, Y. Wada, T. Morioka, Y. Suzuki, F. Gejyo, M. Arakawa, and T. Oite. 2001. Real-time observation of hemodynamic changes in glomerular aneurysms induced by anti-Thy-1 antibody. *Kidney Int*. 59:252-9.

13. Roman, R.J., P.K. Carmines, R. Loutzenhiser, and J.D. Conger. 1991. Direct studies on the control of the renal microcirculation. *J Am Soc Nephrol*. 2:136-49.

14. Saito, M., S. Homma, I. Yamatsu, M. Sato, and N. Ohshima. 1994. Visualization of renal microcirculation in isolated Munich-Wistar rat kidneys: effects of endothelin-1 on renal hemodynamic activity. *Jpn J Pharmacol.* 66:221-9.

15. Schnackenberg, C.G., and C.S. Wilcox. 2001. The SOD mimetic tempol restores vasodilation in afferent arterioles of experimental diabetes. *Kidney Int*. 59:1859-64.

16. Thomson, S.C., A. Deng, D. Bao, J. Satriano, R.C. Blantz, and V. Vallon. 2001. Ornithine decarboxylase, kidney size, and the tubular hypothesis of glomerular hyperfiltration in experimental diabetes. *J Clin Invest*. 107:217-24.

17. Veelken, R., K.F. Hilgers, A. Hartner, A. Haas, K.P. Bohmer, and R.B. Sterzel. 2000. Nitric oxide synthase isoforms and glomerular hyperfiltration in early diabetic nephropathy. *J Am Soc Nephrol*. 11:71-9.

18. Yamamoto, T., K. Hayashi, H. Matsuda, Y. Tomura, Y. Ogasawara, R. Hashimoto, T. Tada, H. Tanaka, and F. Kajiya. 2000. Direct in vivo visualization of glomerular microcirculation by intravital pencil lens-probe CCD videomicroscopy. *Clin Hemorheol Microcirc*. 23:103-8.

19.Yamamoto, T., Y. Tomura, H. Tanaka, and F. Kajiya. 2001. In vivo visualization of characteristics of renal microcirculation in hypertensive and diabetic rats. *Am J Physiol Renal Physiol*. 281:F571-7.

注：本研究は、2001 年 10 月 16 日『第 34 回米国腎臓学会』と 2002 年 5 月 24 日『第 45 回日本腎臓学会』にて発表。

作成日：2002 年 2 月 7 日