Lipocalin-Type Prostaglandin D Synthase in Essential Hypertension

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Abstract—Lipocalin-type prostaglandin D synthase (L-PGDS) reportedly well predicts cardiovascular injuries in humans. However, little is known about the implications of L-PGDS in hypertension. In the present study, we investigated the alterations of serum and urinary L-PGDS in hypertensive patients with or without renal dysfunction. A total of 111 patients with hypertension (EHT; 65 with normoalbuminuria, 23 with microalbuminuria, 12 with macroalbuminuria, 11 with renal failure) and 102 normotensive, normoalbuminuric subjects (NT) were studied. L-PGDS was measured by enzyme-linked immunosorbent assay, and L-PGDS in the kidney was localized using immunohistochemical methods. Blood pressure was higher in EHT groups than in the NT group (P<0.0001). There were no differences in age, gender, BMI, TC, TG, and HbA1c levels among the groups. Serum creatinine and urinary albumin levels were higher in the group with renal failure. Serum levels of L-PGDS were increased in EHT with normoalbuminuria, as compared with NT (0.88±0.05 versus 0.65±0.02 μg/mL; P<0.001). Serum levels of L-PGDS increased with the renal function worsened and positively correlated with serum creatinine, particularly in patients with renal impairments (r=0.76, P<0.0001). Similarly, the urinary L-PGDS excretions in EHT with normoalbuminuria were higher than that in NT (2.31±0.29 versus 1.16±0.14 mg/gCr, P<0.001), whereas there were no differences in urinary albumin excretion between the 2 groups. Moreover, urinary L-PGDS excretion increased dramatically with an increase in albuminuria or proteinuria. L-PGDS was stained in the tubules and the interstitium of the kidney in nephrosclerosis. In conclusion, patients with hypertension exhibited a higher level of L-PGDS in serum and urine, and this became increasingly obvious along with advance in renal dysfunction. These data suggest that L-PGDS metabolism is related to blood pressure and kidney injuries associated with hypertension. (Hypertension. 2002;39[part 2]:449-454.)

Key Words: prostaglandins ■ hypertension, essential ■ renal injury ■ albuminuria ■ blood pressure

Lipocalin-type prostaglandin D synthase (L-PGDS) is unique in its bifunctional character as an enzyme synthesizing prostaglandin D2 (PGD2) and a secretary protein of the lipocalin superfamily that operate as a carrier protein for small lipophilic molecules such as retinol. L-PGDS was recently identified to be the same protein as β-trace, which was originally discovered in 1961 as a major protein of human cerebrospinal fluid, and PGD2 biosynthesized in the local region is known as a potent endogenous sleep modulator, anticoagulant, and vasodilator.

Recent studies have highlighted the implications of L-PGDS in progression of cardiovascular injuries. L-PGDS is demonstrated to occur in smooth muscle cells with a synthetic phenotype and in atherosclerotic intima and accumulate in atherosclerotic plaque of coronary arteries with severe stenosis. In patients with angina pectoris, plasma L-PGDS concentrations in the great cardiac vein were greater than those in the coronary artery, suggesting that L-PGDS is biosynthesized and secreted from the myocardium or in sclerotic endothelial cells. Furthermore, fluid shear stress was reported to enhance the production of L-PGDS mRNA expression in vascular endothelial cells. In these settings, it is of clinical interest to clarify the relationship between hemodynamics and L-PGDS in a variety of pathophysiological conditions.

Recently, we demonstrated the increased urinary excretions of L-PGDS in patients with the early stage of diabetic nephropathy. The meanings and the roles of L-PGDS in the urine are not clear; however, it is assumed that L-PGDS is related to glomerulotubular injuries of the kidney. In concert
with this, recent studies have revealed that serum L-PGDS is increased in chronic renal failure, and this may be attributable to reduction of glomerular filtration rate in advanced renal dysfunction.\textsuperscript{13,14,15}

Accordingly, in the present study, we report for the first time that serum L-PGDS values and urinary excretions of L-PGDS are much higher in patients with essential hypertension (EHT) than those in normotensive subjects, even when the patients with essential hypertension exhibit apparently normal renal function. Hypertension with renal injuries is associated with further increased L-PGDS concentrations in sera and in urine.

### Methods

#### Study Subjects

We recruited 111 patients with EHT with or without renal involvement (65 without microalbuminuria [HT], 23 with microalbuminuria defined as urinary excretion of albumin >30 mg/gCr and <300 mg/gCr [Micro], 12 with macroalbuminuria defined as urinary excretion >300 mg/gCr [Macro], and 11 with chronic renal failure [CRF]) and 102 normotensive subjects with normal albuminuria (NT). The control subjects were selected from volunteers for regular medical checkups. The diagnosis of EHT was based on the criteria of the sixth report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (JNC VI) through repeated measurements of blood pressure (BP), biochemical and hormonal examinations, and history of the illness. BP was measured by a sphygmomanometer in a sitting position after 10 minutes of rest. All subjects were Japanese, and their relatives were not included in the present study. The subjects had been under hypertensive management in outpatient hospitals, and approximately half of the patients were being treated with oral antihypertensive medicines because of renal involvement at the time of the study. The present study was approved by an institutional ethical committee, and informed consent was obtained from each subject. After the subjects awoke, the urine voided for the first time in the morning was collected to determine L-PGDS concentrations in urine and various parameters indicating kidney function.

#### Measurements

The concentrations of serum and urinary L-PGDS were measured by an enzyme-linked immunosorbent assay using 2 monoclonal antibodies, Mab7F5 and Mab1B7, as described previously.\textsuperscript{16} The intra- and interassay coefficients of variation were 3.2 and 5.7\%, respectively.

Albumin in urine was measured by the standard immunoprecipitation method.\textsuperscript{17} The other biochemical markers were measured with an autoanalyzer. HbA1c was determined by high-performance liquid chromatography.

#### Enzyme Immunohistochemistry of the Human Kidney

We had the opportunity to investigate the localization of L-PGDS in human kidneys. One patient had nephrosclerosis, and 6 patients had renal cancer in a localized area. Using the biopsied specimen or apparently normal part of the removed kidney, we investigated immunohistochemically the localization of L-PGDS in the kidney. We obtained informed consent from these patients to investigate their kidney tissue removed for the present study.

To localize L-PGDS molecules in the kidney immunohistochemically, we used the 2 monoclonal antibodies against human L-PGDS, Mab1B7 and Mab7F5, which recognize different antigenic epitopes of the enzyme, and a rabbit polyclonal antibody raised against recombinant human L-PGDS.\textsuperscript{9,13,18} In brief, deparaffinized sections were digested with 0.3% (wt/vol) pepsin (Sigma-Aldrich Japan) in 0.01 mol/L HCl for 5 minutes at room temperature to unmask the antigens and incubated at 4°C overnight with 4°C antibiant human L-PGDS (1 mg/mL) at 4°C overnight and used as

#### Statistical Analysis

Data are expressed as the mean±SE. To compare clinical characteristics between NT and HT or other group, we used the Tukey-Kramer test after 1-way analysis of variance. Possible predictors of BP and L-PGDS levels in serum and urine were tested by multivariate analysis. Statistical analyses were performed using Stat-View J version 5.0 (SAS Institute Inc). A value of $P<0.05$ was considered to be significant.

#### Results

### Clinical Characteristics of Study Subjects

The Table shows the clinical and laboratory findings of the study subjects. There were no differences in age, gender,
Serum levels of L-PGDS were significantly increased in patients with EHT with various stages of renal injuries. A, the difference of serum L-PGDS between NT and HT (without renal involvement determined by albuminuria). B, the differences of serum L-PGDS among various groups that participated in this study (with or without renal involvement). C, the relationship between serum Cr ($x$ axis) and serum levels of L-PGDS ($y$ axis) in all subjects. *$P<0.001$ versus NT; †$P<0.01$ versus HT.

L-PGDS Levels in Serum and Urine

Serum levels of L-PGDS were significantly increased in HT compared with NT (0.88±0.05 versus 0.65±0.02 μg/mL; $P<0.001$; Figure 1A). Furthermore, serum levels of L-PGDS increased in a parallel direction with progression of the hypertensive renal injuries (Figure 1B). Serum levels of L-PGDS were positively correlated with serum Cr, particularly in patients with renal impairments ($r=0.76$, $P<0.0001$; Figure 1C). This relationship was also recognized even when the CRF group was excluded ($r=0.43$, $P<0.0001$ [n=202]). Moreover, we analyzed the data from NT and HT groups alone and found the same correlation, although the coefficient value was lower ($r=0.26$, $P<0.0008$; n=167).

The urinary L-PGDS excretions in HT were also increased, as compared with NT (2.31±0.29 versus 1.16±0.14 mg/gCr; $P<0.001$; Figure 2A). However, there was no difference in urinary excretions of albumin between HT and NT (10.7±0.8 versus 9.3±0.7 mg/gCr; $P=NS$). In HT and NT, urinary excretions of L-PGDS were positively correlated with serum L-PGDS ($r=0.49$, $P<0.0001$; Figure 2B). Urinary L-PGDS excretions increased dramatically along with exacerbation of albuminuria or proteinuria (Figure 2C), and its overall excretions were also correlated with serum L-PGDS concentrations ($r=0.85$, $P<0.0001$; Figure 2D).

Multivariate Analysis

We assessed these correlations by using multivariate analysis. The parameters include mean BP, age, gender, BMI, serum L-PGDS, Cr, total cholesterol, triglyceride, high-density lipoprotein cholesterol, urinary excretions of L-PGDS and albumin, and HbA1c levels. In NT and HT groups, the independent determinants of mean BP were urinary excretions of L-PGDS ($P<0.05$), serum L-PGDS ($P<0.05$), and gender ($P<0.05$). The independent determinants of serum L-PGDS were urinary excretions of L-PGDS ($P<0.005$) and serum Cr ($P<0.005$), mean BP ($P<0.05$), and gender ($P<0.05$). The urinary excretions of L-PGDS were determined independently by serum L-PGDS ($P<0.005$), mean BP ($P<0.05$), and urinary excretions of albumin ($P<0.05$).

Localization of L-PGDS in the Kidney by Immunohistochemical Technique

We attempted to localize L-PGDS in the kidney using immunohistochemistry. In normal subjects, L-PGDS molecules were stained in the interstitium of proximal tubules in Figure 3A (arrowheads). The tubules and basement membranes per se were negative for the enzyme, and the L-PGDS molecules were not present in the mesangial area. The positive staining of the renal tissues was completely blocked by applying a large amount of standard L-PGDS, as indicated in the graph interposed in Figure 3B, thereby suggesting that we stained indeed L-PGDS enzyme in the kidney. In the renal tissue of nephrosclerosis, the L-PGDS was stained much in the tubules, especially proximal tubules (arrows), and relatively weaker staining than normal subjects was observed in the interstitium (arrowheads), as indicated in Figures 3C and 3D.

Discussion

In the present study, we examined the serum levels and urinary excretions of L-PGDS in NT and EHT. Even in groups without renal injuries, serum levels of L-PGDS were significantly higher in HT than in NT. Furthermore, multivariate analysis revealed that mean BP is an independent determinant of serum L-PGDS levels and urinary excretions of L-PGDS. In this study, we also demonstrated that there were relationships between the renal injuries and the serum levels or urinary excretions of L-PGDS, which is in accordance with the data reported previously. 

The origin or the role of serum L-PGDS is not fully understood. However, the major source of L-PGDS is presumed to be vascular endothelium. Indeed, fluid shear stress induces L-PGDS expression in vascular endothelial cells, and PGD$_2$ and 15d-PGJ$_2$ are released into culture.
medium. It is also reported that, in patients who have angina pectoris and are treated with percutaneous transluminal coronary angioplasty, serum L-PGDS in the coronary sinus transiently decreases and the subsequent alteration is a good predictor of the subsequent restenosis. They have demonstrated in their study that the patients whose serum L-PGDS remains at baseline levels likely exhibit coronary restenosis in the follow-up study. Meanwhile, L-PGDS is expressed in a synthetic phenotype of smooth muscle cells and in the atherosclerotic intima and accumulates in the atherosclerotic plaque of coronary arteries with severe stenosis. These data favor the hypothesis that L-PGDS works against vascular injury. Indeed, it is reported that PGD2 generated through L-PGDS inhibits inducible nitric oxide expression after cytokine stimulation both in smooth muscle cells and in endothelial cells. On the basis of our preliminary studies, L-PGDS is expressed in cardiovascular lesions or in the proximal tubules in patients with diabetes. In addition, we recently found that L-PGDS is overexpressed in arterial smooth muscle cells in patients with diabetes. Considering these data, we presume that L-PGDS expressed in the injured organ behaves as a sort of adaptation mechanism.

In patients with EHT, we demonstrated that the urinary excretions of L-PGDS were determined solely by high BP (Figure 2A). As seen in albuminuria, L-PGDS excretions strikingly increased along with progression of renal injuries (Figure 2C). In this context, we previously reported that urinary excretions of L-PGDS in diabetic nephropathy increased with progression of renal injuries. The precious mechanisms of increased excretions of L-PGDS in urine are not yet clarified. However, we speculate that they are due to increased filtration from the glomeruli after the increase in serum levels of L-PGDS or increased production at tubules. Indeed, urinary excretions of L-PGDS were positively correlated with serum L-PGDS level, and renal histology demonstrated occurrence of L-PGDS antigenicity at tubules and interstitium of the kidney (Figures 2 and 3).

**Figure 2.** Urinary excretions of L-PGDS in hypertensive patients with each stage of renal injuries. A, the difference in urinary L-PGDS between NT and HT (without renal involvement determined by albuminuria). B, the relationship between serum L-PGDS (x axis) and urinary levels of L-PGDS (y axis) in NT and HT. C, the differences in urinary excretions of L-PGDS in various groups recruited in this study. D, the relationship between serum L-PGDS (x axis) and urinary excretions of L-PGDS (y axis) in all subjects. *P<0.001 versus NT; #P<0.01 versus HT.

**Figure 3.** Photomicrographs of the kidney stained for L-PGDS by Mab-1B7 antibody. In the kidney of NT without sclerosis, L-PGDS was stained in the interstitium of peritubular area (arrowheads). The tubules were negative for staining (A, ×200). The L-PGDS staining in the kidney was completely blocked by applying a large amount of standard L-PGDS (B). L-PGDS was localized in the tubules (arrow), and a weaker signal of L-PGDS antigenicity was shown in the interstitium (arrowhead) of the kidney of nephrosclerosis (C and D). The other forms of the anti-L-PGDS antibody, Mab7F5, and the polyclonal antibody also showed the same localization of L-PGDS.
The roles of L-PGDS at the kidney are not clarified. Intrarenal infusion of PGD2 resulted in a dose-dependent increase in renal arterial flow, urine output, Cr clearance, and sodium and potassium excretion in dog.20 Recently, substantial amounts of prostaglandin H2, a precursor of PGD2, was shown to be released from rat glomeruli and glomerular mesangial cells.21 PGD2 is also detected at the incubation medium of mesangial cells after stimulation by arachidonic acid.21 Furthermore, PGJ2, a metabolite of PGD2, inhibits inducible nitric oxide expression in mesangial cells.22 These data suggest the possible roles of L-PGDS in the glomerular hemodynamics and function. However, the localization of prostaglandin D2 receptor in the kidney has not yet been determined.23,24 The precise mechanism of L-PGDS actions in renal pathophysiology remains to be elucidated.

The molecular weight of L-PGDS is 26 000 Da, which varies depending on the size of the glycosyl moiety.25 This enzyme is one of the so-called low-molecular-weight proteins, which can pass through the membrane sieve of the glomeruli. Low-molecular-weight proteins, including retinol-binding protein (RBP), β2-microglobulin, and α1-microglobulin, are known as markers that indicate injuries in the renal tubules. The renal proximal tubule exhibits a very extensive apical endocytic apparatus consisting of an elaborate network of coated pits and small coated and noncoated endosomes. This endocytic apparatus having megalin is involved in the reabsorption of molecules filtered in the glomeruli.26–28 L-PGDS is a secretory protein of the lipocalin superfamily that operates as a carrier protein for small lipophilic molecules such as retinol.1,2 Because the function and the molecular weight of L-PGDS are similar to RBP, it seems possible that L-PGDS is reabsorbed at proximal tubules like RBP in the kidney. Indeed, we revealed the presence of L-PGDS in the renal tubules and in the interstitium by immunohistochemistry (Figure 3). Furthermore, in a patient with nephro-sclerosis, tubular staining for L-PGDS was augmented compared with normal subjects, suggesting that increased uptake or production of L-PGDS might reflect an increase in L-PGDS biosynthesis in essential hypertension.

We preliminarily investigated the effects of short-term treatment with the ARB losartan (n = 10) and the Ca channel blocker amlodipine (n = 10). Three months’ treatments did not influence the urinary excretions of L-PGDS (unpublished data). These data suggest that at least short-term treatments did not influence L-PGDS excretions in urine and that direct effects of antihypertensive drugs could be neglected.

In conclusion, we demonstrated that serum and urinary levels of L-PGDS were increased in essential hypertension, even in normal renal function. The increase in serum L-PGDS was associated with BP, urinary excretions of L-PGDS, serum Cr, and gender. Urinary L-PGDS preceded an increase in urinary microalbuminuria and was related to urinary albumin excretion. This increase probably reflects injuries in the renal tubules and arterioles induced by hypertension. These data strongly suggest that BP, L-PGDS, and renal function are closely interrelated. It is worthwhile to investigate the pathophysiological and diagnostic implications of L-PGDS in hypertension and the related organ injuries in humans.

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References


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