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Renal protective effect of YM598, a selective endothelin ET_A receptor antagonist, against diabetic nephropathy in OLETF rats

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Abstract

We have investigated the effect of potassium (*E*)-*N*-[6-methoxy-5-(2-methoxyphenoxy)-2-(pyrimidin-2-yl) pyrimidin-4-yl]-2-phenylenthenesulfonamidate (YM598), a selective endothelin ET_A receptor antagonist, on renal function in Otsuka Long–Evans Tokushima Fatty (OLETF) rats, an animal model of type II diabetes. YM598 (0.1 or 1 mg kg^{-1}), enalapril (5 mg kg^{-1}), an angiotensin-converting enzyme inhibitor, or vehicle was administered once daily by gastric gavage to 22-week-old male Otsuka Long–Evans Tokushima Fatty rats for 32 weeks. Enalapril but not YM598 mildly lowered blood pressure in the diabetic rats. YM598 blunted the development of albuminuria in a dose-dependent manner. High dose of YM598 reduced albuminuria comparable to enalapril. Urinary endothelin-1 excretion was greater in the diabetic than in the control rats, and was not substantially influenced by the agents. These data suggest that endothelin is involved in the progression of diabetic nephropathy in Otsuka Long–Evans Tokushima Fatty rats, and an endothelin ET_A receptor antagonist may be useful for the treatment of diabetic nephropathy.

Keywords: Diabetic, rat; Endothelin; Endothelin ET_A receptor antagonist; Diabetic nephropathy

1. Introduction

Diabetic nephropathy is becoming the most frequent single cause of end-stage renal disease in many industrial countries. Although much effort was paid to treat the complication (Ohkubo et al., 1995; The Microalbuminuria Captopril Study Group, 1996), the prognosis of diabetic patients is still poor.

The status of endothelin-1 plasma concentrations in patients with type I as well as type II diabetes mellitus is controversial (Hopfner and Gopalakrishnan, 1999). However, increased urinary excretion of endothelin-1 has been reported in patients with nondiabetic renal diseases (Ohta et al., 1991; Vlachojannis et al., 1997) and diabetic nephropathy (Lee et al., 1994; Shin et al., 1996). In the streptozotocin-induced diabetic rats, the expression of endothelin-1 messenger RNA is enhanced in renal glomeruli (Fukui et al., 1993). In addition, the endothelin type A (ET_A) and type A and B ($ET_{A/B}$) receptor antagonists protect against the progression of diabetic nephropathy in these chemical-induced diabetic animals (Hocher et al., 1998; Benigni et al., 1998). These data indicate that endothelin-1 is a contributory mediator of renal damage in the streptozotocin-treated rats, a model of type I diabetes mellitus.

Otsuka Long–Evans Tokushima Fatty (OLETF) rat is a newly developed model of human type II diabetes mellitus characterized by late-onset of hyperglycemia, the mild and chronic course of diabetes mellitus, and the association of diabetic complications (Kawano et al., 1992). We previously demonstrated that albuminuria is already detected at 22 weeks of age, and enalapril, an angiotensin-converting enzyme inhibitor, ameliorates the nephropathy in these spontaneous diabetic animals (Sugimoto et al., 1999).

The aim of the present study was to compare the renoprotective effects of an orally active endothelin ET_A receptor antagonist, potassium (*E*)-*N*-[6-methoxy-5-(2- methoxyphenoxy)-2-(pyrimidin-2-yl) pyrimidin-4-yl]-2-phenylenthenesulfonamidate (YM598) (Harada et al., 2001), and enalapril. OLETF rats at 22 weeks of age began to receive the agents for 32 weeks. Not only urine endothelin-1 and albumin, but urine type IV collagen and heparan sulfate, the markers for early diabetic nephropathy (Gambaro et al.,

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1989; Hayashi et al., 1992; Kado et al., 1996), were measured.

2. Materials and methods

2.1. Animals

Male OLETF and Long–Evans Tokushima Otsuka (LETO) rats were kindly supplied by the Tokushima Research Institute (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan). All rats were kept in a room under the specific pathogen-free environment with controlled temperature, humidity and a 12-h light/dark cycle. Animals were housed two per cage and were free access to standard rat chow (CA-1; Japan Clea Co., Ltd., Tokyo, Japan) and water before and during the study. After the basal measurements, OLETF rats were randomly divided into four groups ($n=10$ in each group); group I with vehicle (distilled water) alone, group II with enalapril maleate at daily dose of 5 mg kg^{-1} body weight, and groups III and IV with YM598 at daily doses of 0.1 and 1 mg kg^{-1} body weight, respectively. YM598, a newly developed orally active endothelin type A (ET_A) receptor antagonist, was supplied by Yamanouchi Pharmaceutical Co., Ltd. (Tokyo, Japan). The oral doses of YM598 more than 0.1 mg kg^{-1} body weight to rats suppressed the pressor response to exogenously administered endothelin-1 in a dose-dependent manner (Sanagi et al., 1998). Maximum antagonism was noted at 1 mg kg^{-1} body weight of YM598 and the effect lasted for 24 h (Sanagi et al., 1998). Enalapril maleate was obtained from Sigma Chemical Co. (St. Louis, MO, USA). The dose of enalapril was selected based on the previous study showing its renoprotective effect in OLETF rats (Sugimoto et al., 1999). The agent dissolved in distilled water was administered once daily by gastric gavage. The treatment was initiated at 22 weeks of age and continued for the following 32 weeks. At 22 weeks of age, diabetes mellitus were already established in these animals (Fukuzawa et al., 1996). LETO rats ($n=10$) were used as a control and received vehicle. The

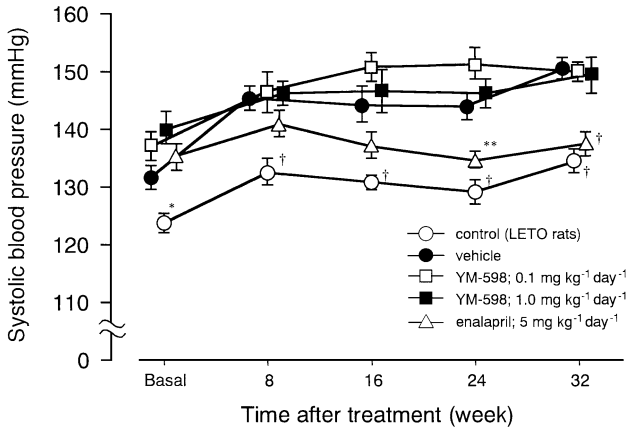

Fig. 1. Systolic blood pressure in LETO and OLETF rats treated for 32 weeks starting at 22 weeks of age. $n=10$ in each group. $*P<0.05$, $**P<0.01$, $\dagger P<0.001$ vs. the vehicle-treated OLETF rats.

Table 1
Changes in body weight, plasma glucose and serum insulin concentrations in OLETF and LETO rats

	OLETF rats				LETO rats
	Vehicle	Enalapril (5 mg/kg/day)	YM-598 (0.1 mg/kg/day)	YM-598 (1 mg/kg/day)	
Body weight (g)					
Before	556 ± 9	563 ± 10	534 ± 5	525 ± 10	420 ± 4[a]
8 weeks	660 ± 10	648 ± 12	653 ± 6	637 ± 12	483 ± 6[a]
16 weeks	696 ± 9	685 ± 15	690 ± 7	677 ± 12	515 ± 7[a]
24 weeks	723 ± 8	695 ± 12	707 ± 9	695 ± 15	533 ± 6[a]
32 weeks	726 ± 9	697 ± 14	718 ± 7	699 ± 14	549 ± 5[a]
Plasma glucose (mg/dl)					
Before	168 ± 5	163 ± 4	165 ± 11	153 ± 3	126 ± 3[a]
8 weeks	205 ± 9	209 ± 12	213 ± 9	187 ± 7	145 ± 5[a]
16 weeks	212 ± 11	204 ± 8	204 ± 6	185 ± 5	125 ± 3[a]
24 weeks	209 ± 4	193 ± 7	197 ± 7	201 ± 11	130 ± 3[a]
32 weeks	213 ± 10	207 ± 9	200 ± 5	199 ± 4	134 ± 2[a]
Serum insulin (mU/ml)					
Before	29.6 ± 1.9	27.6 ± 1.6	28.9 ± 2.1	28.3 ± 2.4	15.0 ± 1.1[a]
8 weeks	30.9 ± 3.3	36.5 ± 2.7	30.6 ± 1.9	41.5 ± 4.8	11.6 ± 0.9[a]
16 weeks	22.0 ± 2.4	23.3 ± 3.9	26.1 ± 3.0	23.7 ± 2.5	9.4 ± 1.4[a]
24 weeks	30.3 ± 2.8	24.2 ± 3.5	25.6 ± 2.4	22.5 ± 1.9	12.2 ± 1.2[a]
32 weeks	16.3 ± 1.3	22.2 ± 2.4	18.1 ± 0.8	17.4 ± 1.6	7.9 ± 2.0[b]

$n=10$ in each group.
[a] $P<0.001$ vs. the vehicle-treated OLETF rats.
[b] $P<0.01$ vs. the vehicle-treated OLETF rats.

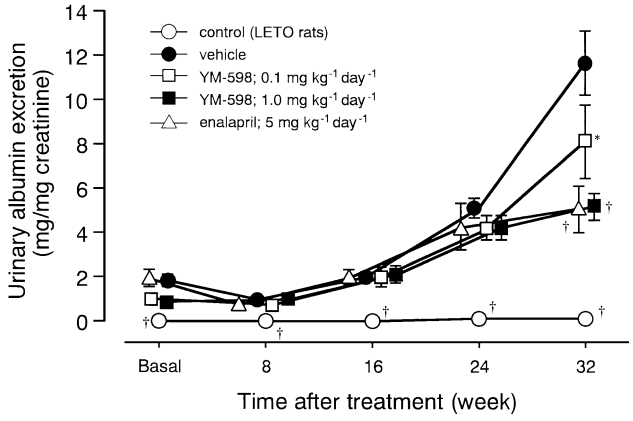

Fig. 2. Urine albumin excretion in LETO and OLETF rats treated for 32 weeks starting at 22 weeks of age. $n=10$ in each group. $*P<0.05$, $**P<0.01$, $\dagger P<0.001$ vs. the vehicle-treated OLETF rats.

dose of agent was adjusted by body weight measured twice a week.

Blood pressure, blood chemistries, urine excretions of albumin, type IV collagen, endothelin-1 and heparan sulfate, and endogenous creatinine clearance were measured before and during the treatment period with an 8-week interval. Blood sample was taken from the tail vein at post-prandial period (around the period of light on). The experiments were conducted in accordance with the Jichi Medical School Guide for Laboratory Animals.

2.2. Blood pressure measurement and urine collection

Blood pressure was measured in prewarmed, awake rats by a standard tail-cuff method (KN-210-1, Natsume Co., Ltd., Tokyo, Japan). At least 15 determinations were made and serial lowest 3 measurements were averaged to obtain a mean value of systolic blood pressure for each rat.

To determine urinary excretions of albumin, type IV collagen, endothelin-1 and heparan sulfate, and creatinine clearance, urine was collected for 4 h in metabolic cages following water loading (2% of body weight) by gastric gavage. Urine excretions of these variables were normalized in terms of urine creatinine concentrations.

2.3. Blood and urine chemistries

Urinary concentrations of albumin were measured by enzyme-linked immunosorbent assay kit (NEPHRAT II; Exocell Inc., Philadelphia, PA, USA). Urinary type IV collagen was determined by sandwich enzyme immunoassay (EIA) kit (Tahara International Laboratories, Inc., Tsukuba, Japan) and urinary endothelin-1 by sandwich EIA kit (Immuno Biological Laboratories, Fujioka, Japan). Urine heparan sulfate concentration was measured by the method of Van den Lest et al. (1994). Serum cholesterol and creatinine, and urinary creatinine were measured by an autoanalyzer (Hitachi 7170, Hitachi; Co., Ltd., Tokyo, Japan). Plasma glucose was determined by the glucose oxidase method (Lott and Turner, 1975). Serum insulin concentration was determined by a radioimmunoassay kit (Linco Research Inc., St. Charles, MO, USA).

2.4. Statistical analysis

Results are expressed as the means ± S.E. The statistical significance was tested by two-way ANOVA on repeated measurements and post hoc test (Fisher's protected least significant difference test) using StatView J-4.02 (Abacus Concepts Inc., Berkeley, CA, USA). A P less than 0.05 was considered statistically significant.

Table 2
Changes in urine excretions of heparan sulphate and collagen type IV in OLETF and LETO rats

	OLETF rats				LETO rats
	Vehicle	Enalapril (5 mg/kg/day)	YM-598 (0.1 mg/kg/day)	YM-598 (1 mg/kg/day)	
Heparan sulphate (mg/mg creatinine)					
Before	55.2 ± 23.2	42.6 ± 9.5	41.1 ± 11.8	33.7 ± 4.7	35.5 ± 7.4
8 weeks	98.0 ± 10.5	60.3 ± 14.8[a]	57.7 ± 10.8[a]	44.5 ± 6.2[b]	33.2 ± 12.2[c]
16 weeks	45.7 ± 7.3	40.0 ± 7.9	46.8 ± 10.0	25.8 ± 3.8[a]	18.6 ± 3.1[b]
24 weeks	63.0 ± 21.6	45.4 ± 8.2	37.1 ± 8.7	39.2 ± 7.6	28.6 ± 3.9[a]
32 weeks	93.1 ± 19.5	45.9 ± 11.6	48.3 ± 10.9[b]	24.9 ± 4.1[c]	37.9 ± 6.6[b]
Type IV collagen (ng/mg creatinine)					
Before	441 ± 69	410 ± 46	372 ± 61	292 ± 29[a]	296 ± 31[a]
8 weeks	544 ± 150	449 ± 88	374 ± 72	513 ± 99	236 ± 59[a]
16 weeks	374 ± 96	166 ± 28[b]	186 ± 27[a]	271 ± 51	203 ± 27[a]
24 weeks	361 ± 58	191 ± 28[b]	197 ± 37[b]	198 ± 33[b]	191 ± 36[b]
32 weeks	379 ± 82	275 ± 51	189 ± 37[a]	235 ± 50	164 ± 19[b]

$n=10$ in each group.
[a] $P<0.05$ vs. the vehicle-treated OLETF rats.
[b] $P<0.01$ vs. the vehicle-treated OLETF rats.
[c] $P<0.001$ vs. the vehicle-treated OLETF rats.

3. Results

3.1. Blood pressure and body weight

All animals survived and completed the study. Body weight in OLETF rats was greater than that in LETO rats ($P<0.0001$). YM598 or enalapril did not affect body weight in OLETF rats (Table 1).

Fig. 1 shows the changes in blood pressure in OLETF and LETO rats. Systolic blood pressure in OLETF rats with vehicle was significantly higher than that in LETO rats during the treatment period ($P<0.0001$). Enalapril mildly but significantly lowered systolic blood pressure ($P<0.01$), especially at 24 and 32 weeks. At doses studied, YM598 did not lower blood pressure in OLETF rats.

3.2. Renal function

In this study, urine albumin excretion was quantitated by urine albumin/creatinine ratio in the 4-h urine following water loading. In OLETF rats, the elevation in urinary albumin excretion was already detected at the initiation of the treatment (Fig. 2). From 16 weeks, urine albumin excretion increased in OLETF rats. Enalapril significantly reduced albuminuria at the end of the treatment ($P<0.01$). YM598 also significantly ($P<0.01$) attenuated the development of albuminuria in a dose-dependent manner.

Urinary excretions of heparan sulfate and type IV collagen were significantly ($P<0.001$) greater in OLETF than in LETO rats (Table 2). Enalapril significantly reduced these parameters ($P<0.05$). YM598 also lowered urinary excretions of heparan sulfate and type IV collagen dose-independently.

3.3. Creatinine clearance

Before the treatment, creatinine clearance in OLETF rats was significantly greater than that in LETO rats (Fig. 3). From 8 weeks, creatinine clearance declined to a comparable level to LETO rats. YM598 or enalapril did not affect the changes in creatinine clearance in OLETF rats.

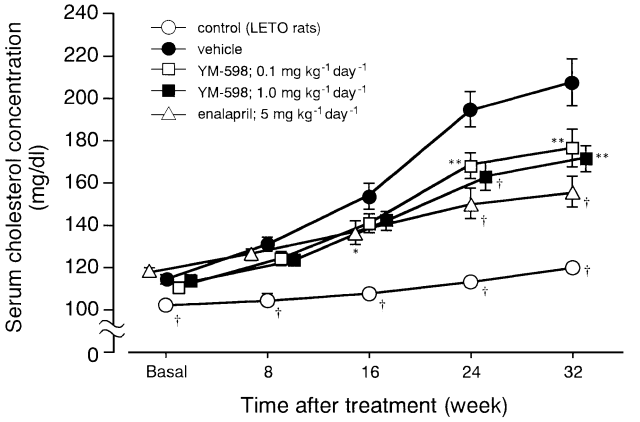

Fig. 4. Serum total cholesterol in LETO and OLETF rats treated for 32 weeks starting at 22 weeks of age. $n=10$ in each group. $*P<0.05$, $**P<0.01$, $\dagger P<0.001$ vs. the vehicle-treated OLETF rats.

3.4. Blood chemistries

Post-prandial plasma glucose in OLETF rats was around 200 mg/dl while that in LETO rats was around 130 mg/dl (Table 1). Serum immunoreactive insulin concentrations in OLETF rats were significantly higher than those in LETO rats during the study. YM598 or enalapril did not affect plasma glucose or serum immunoreactive insulin concentrations in OLETF rats (Table 1). Serum cholesterol in the vehicle-treated OLETF rats remarkably increased during the treatment period (Fig. 4). Enalapril ($P<0.001$) and YM598 ($P<0.01$) significantly attenuated the increase in this variable.

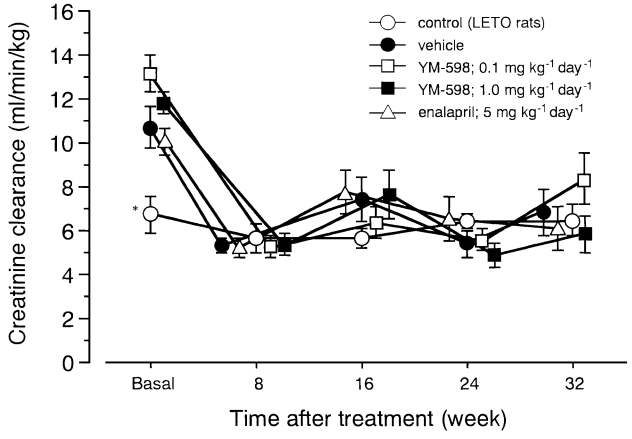

Fig. 3. Creatinine clearance in LETO and OLETF rats treated for 32 weeks starting at 22 weeks of age. $n=10$ in each group. $*P<0.05$, $\dagger P<0.001$ vs. the vehicle-treated OLETF rats.

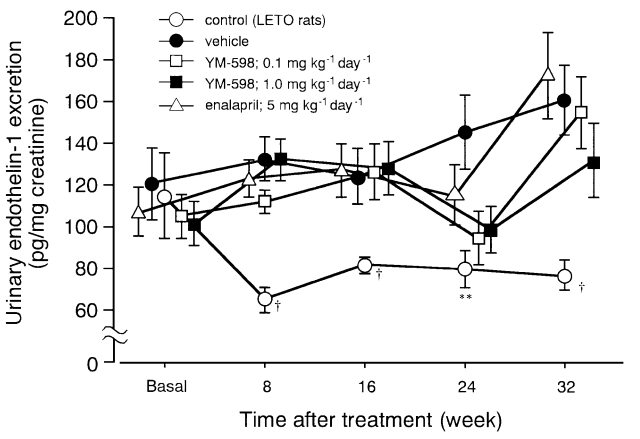

Fig. 5. Urinary endothelin-1 excretion in LETO and OLETF rats treated for 32 weeks starting at 22 weeks of age. $n=10$ in each group. $*P<0.05$, $**P<0.01$, $\dagger P<0.001$ vs. the vehicle-treated OLETF rats.

3.5. Urinary endothelin-1 excretion

Urinary endothelin-1 excretion in OLETF rats with vehicle was significantly greater than that in LETO rats ($P < 0.01$) (Fig. 5). Enalapril or YM598 did not significantly affect urinary endothelin-1 excretion.

4. Discussion

The present study was undertaken to evaluate a potential renoprotective effect of YM598, a selective endothelin ET_A receptor antagonist, in OLETF rats, a model of human type II diabetes mellitus.

Because YM598 or enalapril did not affect the changes in body weight, plasma glucose or serum insulin, it is unlikely that these agents have some influences on the intake of food or glucose metabolism in OLETF rats. The elevated creatinine clearance, indicating glomerular hyperfiltration, and albuminuria were observed in OLETF rats at 22 weeks of age in this study, which suggest that diabetic nephropathy already existed (Fukuzawa et al., 1996). Similar to enalapril, YM598 reduced urinary albumin in these animals with overt proteinuria. These data are compatible with the idea that YM598 has a renoprotective effect against diabetic nephropathy in OLETF rats. In addition, urinary excretions of heparan sulfate and type IV collagen were also decreased in OLETF rats with YM598. Heparan sulfate and type IV collagen are major constituents of glomerular basement membrane. High urinary excretions of these glomerular extracellular matrices reflect the injury of glomerular basement membrane. In the early phase of diabetic nephropathy, heparan sulfate is dropped out from the glomerular basement membrane and urinary excretion of heparan sulfate is increased. Biosynthesis of heparan sulfate is, however, fall, resulting in the reduction in heparan sulfate in the glomerular basement membrane (Rohrbach et al., 1982). Heparan sulfate confers a negative charge on the glomerular basement membrane and is responsible for glomerular permeability as a charge barrier. The loss of this charge in the glomerular basement membrane is compensated by an enhanced synthesis of basement membrane components including type IV collagen. Enhanced renal production of type IV collagen causes increased urinary excretion of the extracellular matrix. Increased urinary excretions of heparan sulfate and type IV collagen, thus, are employed as the useful markers for early diabetic nephropathy (Gambaro et al., 1989; Hayashi et al., 1992; Kado et al., 1996).

Previous studies have shown that the endothelin ET_A as well as $ET_{A/B}$ receptor antagonist protects against the development of diabetic nephropathy in streptozotocin-treated rats (Hocher et al., 1998; Benigni et al., 1998). Based on these data, we think that endothelin-1 is involved in the mechanism of nephropathy in the insulin-dependent and -independent diabetic rats. Endothelin-1 synthesis is enhanced by several mechanisms such as hyperglycemia and shear stress due to osmotically induced glomerular hyperfiltration in diabetic rats (Yamauchi et al., 1990; Hocher et al., 1997). In the kidney, endothelin-1 is produced by mainly tubular-transepithelial and mesangial cells. Furthermore, excessive protein overloading to tubular epithelial cells induces a dose-dependent increase in the synthesis and release of endothelin-1 in proteinemic nephropathies either of diabetic or nondiabetic origin (Benigni et al., 2000). Endothelin-1 causes not only cell proliferation (Simonson and Herman, 1993; Wang et al., 1994) but stimulates production of extracellular matrix proteins (Ruiz-Ortega et al., 1994). Endothelin-1 produced in the renal tubule cells is mainly secreted toward the interstitium, which may contribute to the progression of diabetic nephropathy through renal interstitial fibrosis (Zoja et al., 1995). Most of endothelin-1 filtered from plasma is subject to degradation by neutral endopeptidase in the proximal tubule, urinary endothelin-1 is, thus, probably of renal origin (Abassi et al., 1993). Hocher et al. (1998) found that urinary endothelin-1 excretion significantly elevates while plasma endothelin-1 concentration does not in streptozotocin-induced diabetic rats, indicating that urinary endothelin-1 excretion mainly reflects the endothelin-1 production in renal tissues. As urinary endothelin-1 excretion increased in OLETF rats in this study, we think that the renal endothelin-1 production was also enhanced in these diabetic animals. YM598, a selective endothelin ET_A receptor antagonist, did not affect the urinary endothelin-1 excretion in OLETF rats. Benigni et al. (1998) have demonstrated that AC-(D)-DIP-(L)-Leu-(L)-Asp-(L)-Ile-(L)-Ile-(L)-TRP (PD142,893), a combined endothelin $ET_{A/B}$ receptor antagonist, remarkably reduced endothelin-1 mRNA at both the glomerular and tubular levels in streptozotocin-induced diabetic rats. These data led them to speculate an autoinduction of endothelin-1 via the ET_B receptor that is documented in rat mesangial cells (Iwasaki et al., 1995) as well as in human endothelial and proximal tubular cells (Saijonmaa et al., 1992; Ong et al., 1995). Since YM598 is a specific endothelin ET_A receptor antagonist, the present finding that YM598 did not reduce the urinary endothelin-1 excretion in OLETF rats is consistent with their hypothesis.

Serum cholesterol concentrations elevated with age, and the increase was markedly greater in OLETF than in LETO rats. Such the increase in OLETF rats is believed to be a compensative production of cholesterol in the liver stimulated by hypoalbuminemia due to loss of albumin into urine. It is well known that hyperlipidemia promotes renal injury (Moorhead et al., 1982; Keane et al., 1991; Kramer-Guth et al., 1996). Therefore, an amelioration of hyperlipidemia by YM598, probably through the reduction of albuminuria, may partly contribute to its renoprotective effect observed in this study.

Blood pressure control is an important determinant in the protection of the deterioration of diabetic nephropathy (Parving et al., 1985, 1987, 1988). Meta-analysis of the renoprotective effects of various antihypertensive agents

revealed that an angiotensin-converting enzyme inhibitor is most potent (Kasiske et al., 1993). In this study, enalapril lowered blood pressure while YM598 at the doses used did not. Based on the present as well as previous data showing that plasma endothelin-1 concentration does not significantly elevate in the streptozotocin-induced diabetic rats (Hocher et al., 1998), we think that the role of plasma endothelin-1 in the elevation of blood pressure is small, if any, in these animals.

In summary, we demonstrated that YM598, a selective endothelin ET_A receptor antagonist, has the protective effect against diabetic nephropathy in OLETF rats. In contrast to enalapril, YM598 at doses used did not lower blood pressure in OLETF rats, suggesting that the renoprotective effects through endothelin ET_A receptor antagonism do not depend on hemodynamic effects. The present data support the idea that endothelin-1 plays some roles in the progression of diabetic nephropathy. Endothelin ET_A receptor antagonism may be a new therapeutic strategy in the treatment of diabetic nephropathy.

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