Regular Article

A Novel TNF- α Converting Enzyme (TACE) Selective Inhibitor JTP-96193 Prevents Insulin Resistance in KK-A^y Type 2 Diabetic Mice and Diabetic Peripheral Neuropathy in Type 1 Diabetic Mice

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Tumor necrosis factor- α (TNF- α) converting enzyme/a disintegrin and metalloproteinase domain-containing protein 17 (TACE/ADAM17) is a key sheddase that releases TNF- α from its inactive precursor and is thought as a new drug target to inhibit TNF- α production. In the present study, pharmacological effects of a novel TACE selective inhibitor, JTP-96193, on type 2 diabetes and diabetic peripheral neuropathy (DPN) as its major complication was examined. Enzyme inhibitory activity of JTP-96193 on TACE and other ADAMs was measured in *in vitro*. High fat-induced obese mice and type 2 diabetic KK-A^y mice were used to evaluate the effect of JTP-96193 on insulin resistance. Finally, streptozotocin (STZ)-induced diabetic mice were treated with JTP-96193 to evaluate the sciatic motor nerve conduction velocities (MNCV). JTP-96193 selectively inhibited human TACE activity with IC₅₀ value of 5.4 nM and showed more than 1800-fold selectivity against other matrix metalloproteinases. In mouse models of obesity and diabetes, JTP-96193 reduced the TNF- α release from the fat tissue and prevented development of diabetes and improved insulin resistance, respectively. Furthermore, JTP-96193 prevented delay of sciatic MNCV without any effects on blood glucose or insulin levels in STZ-induced diabetic mice. TACE inhibitor is effective on insulin resistance and DPN independent from glucose-lowering effect. These pharmacological properties of JTP-96193 may be helpful to treat type 2 diabetes accompanied by its microvascular complications.

Key words tumor necrosis factor- α (TNF- α); TNF- α converting enzyme (TACE); diabetes; insulin resistance; diabetic peripheral neuropathy

INTRODUCTION

Obesity is the common metabolic disease and is recognized as a major risk factor of insulin resistance. The population of obese people is increasing all over the world today. Expression of tumor necrosis factor (TNF)- α , an inflammatory adipocytokine produced by macrophages that negatively regulates insulin receptor signal pathway in muscle and fat,¹⁾ is increased in obese humans and animals. Today, five TNF- α inhibitors (etanercept, infliximab, adalimumab, certolizumab pegol, and golimumab) are approved by the U.S. Food and Drug Administration (FDA) and in other countries for the treatment of ankylosing spondylitis, psoriatic arthritis, rheumatoid arthritis (RA), *etc.* All these drugs selectively bind to TNF- α and inhibit its interaction with the TNF receptor.

TNF- α is synthesized as a membrane-anchored precursor and the ectodomain of TNF- α is shed by TNF- α converting enzyme/a disintegrin and metalloproteinase domain-containing protein 17 (TACE/ADAM17) to secrete soluble TNF- α .^{2,3)} Although the function of TACE has not been fully elucidated, it functions as sheddase towards many inflammation-related molecules other than TNF- α , such as transforming growth factor (TGF)- α , heparin binding-epidermal growth factor-like growth factor (HB-EGF), TNF receptors, interleukin (IL)-1 receptor 2, tropomyosin receptor kinase (Trk) A receptor, vas-

cular endothelial growth factor (VEGF) receptor, L-selectin, and vascular cell adhesion molecule (VCAM). Thus, inhibiting TACE activity is expected not only bring higher potential for treating diseases that are targets of TNF- α inhibitors such as RA, but also as a new approach to treat multiple diseases, including obesity-induced insulin resistance and type 2 diabetes.⁴⁾

Recently, in addition to hypoglycemic effect, anti-diabetic drugs are highly required to provide widespread additive value on their pharmacological properties. Glucagon-like peptide-1 (GLP-1) analogs such as liraglutide and sodium glucose co-transporter-2 (SGLT-2) inhibitors such as canagliflozin, show lower rate of cardiovascular events compared to placebo treatment in type 2 diabetes patients.^{5,6)} SGLT-2 inhibitor also delays the progression of kidney disease and prevents the clinical renal events.⁷⁾ Effects of TNF- α inhibitors on diabetic peripheral neuropathy (DPN)^{8,9)} and diabetic nephropathy¹⁰⁾ have been reported in diabetic animal models.

In the present study, we synthesized a novel TACE inhibitor, JTP-96193, and evaluated its effects on insulin resistance in animal models of insulin resistance and type 2 diabetes. Further potential of JTP-96193 on DPN in streptozotocin (STZ)-induced diabetic mice was also evaluated, because whether TACE inhibitor prevents the development of diabetic microvascular complications as an additional pharmacological

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property has not been reported.

MATERIALS AND METHODS

Animals and Chemicals Male Lewis rats, C57BL/6J mice, and ICR mice were purchased from Charles River Laboratories Japan (Yokohama, Japan). KK-A^y mice were purchased from CLEA Japan (Tokyo, Japan). All animal protocols complied with our Laboratory Guidelines for Animal Experimentation. Animals were housed in a controlled room (temperature $23 \pm 3^\circ\text{C}$, humidity $55 \pm 15\%$, 12 h lighting cycle) and allowed free access to diet and water. Animals were fed normal diet (CRF-1), 35% high-fat diet (HFD), or 3.1% low-fat diet (LFD) (Oriental Yeast; Tokyo, Japan).

JTP-96193 (molecular weight: 525.6), a thiadiazolone-derivative TACE inhibitor, was synthesized at Central Pharmaceutical Research Institute, Japan Tobacco, Inc. (Osaka, Japan).

TACE Enzyme Assay Enzyme activity of TACE was measured with some modifications of method reported by Patel *et al.*¹¹⁾ The TNF- α -specific peptide substrate (MCA-Pro-Leu-Ala-Gln-Ala-Val-Dpa-Arg-Ser-Ser-Ser-Arg-NH₂, Calbiochem; San Diego, CA, U.S.A.) was diluted to a final concentration of $20 \mu\text{M}$ in a buffer containing 25 mM Tris-HCl, $25 \mu\text{M}$ ZnCl₂, 0.005% Brij35 (pH 9.0). The enzyme reaction contained $150 \mu\text{L}$ of a recombinant human TACE (R&D Systems; Minneapolis, MN, U.S.A.) plus the diluted peptide in $300 \mu\text{L}$ final volume. This was incubated for 1 h on an orbital shaker at 25°C . Reactions were quenched by adding $250 \mu\text{L}$ ethylenediaminetetraacetic acid (EDTA) (58 mM), and plates were read at 325 nm excitation/405 nm emission.

Whole Blood Assay Whole blood obtained from male Lewis rats was used for *in vitro* whole blood assay. JTP-96193 or dimethyl sulfoxide (DMSO) ($10 \mu\text{L}$) and $80 \mu\text{L}$ of whole blood were mixed on a 96 well microplate and incubated for 30 min at 37°C . Then, $10 \mu\text{L}$ of $50 \mu\text{g/mL}$ lipopolysaccharide (LPS) was added to the mixture and the microplate was further incubated for 2 h. Samples were centrifuged to collect plasma and TNF- α concentrations were measured using TNF- α enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems).

In vivo whole blood assay was also performed using male Lewis rats. Rats were divided into four groups, to which vehicle (0.5% methyl cellulose) or JTP-96193 (1, 3, or 10 mg/kg) was orally administered. One hour after treatment, $10 \mu\text{g/kg}$ LPS was intravenously injected *via* tail vein to induce systemic TNF- α release. Plasma was collected 1 h after LPS injection and TNF- α concentration was measured using TNF- α ELISA kit.

Epididymal Fat TNF- α and Non-esterified Acid (NEFA) in Diet-Induced Obesity (DIO) Mice Five-week-old C57BL/6J mice were fed HFD for 6 weeks to induce obesity. Animals were divided into three groups based on body weight gain, cumulative food consumption, and blood parameters (glucose, triglyceride (TG), total cholesterol (TC), NEFA, and insulin) after obesity was established. The first group was fed basic HFD for 4 weeks from 12 weeks of age. The second and third groups were fed HFD containing 3 or 30 mg/kg/d JTP-96193, respectively.

After 4 weeks of treatment with JTP-96193, mice were anesthetized with isoflurane and the blood was collected from abdominal aorta. Thereafter, animals were euthanized and

epididymal fat tissue was isolated. Approximately 100 mg of epididymal fat was put into $500 \mu\text{L}$ of Krebs-Ringer Bicarbonate Buffer (KRB) with 100 ng/mL LPS and incubated for 4 h at 37°C . Supernatants were collected to measure TNF- α concentrations using TNF- α ELISA kit and NEFA concentrations using NEFA-C test Wako (FUJIFILM Wako Pure Chemical Corporation; Osaka, Japan).

Blood Parameters in Diabetic KK-A^y Mice KK-A^y mice were prepared to evaluate insulin-sensitizing effect of TACE inhibitor. Twenty-four mice were divided into three groups: the first group was fed normal diet (CRF-1), the second and third groups were fed JTP-96193 admixture diet at 3 and 30 mg/kg/d , respectively, for 2 weeks. Plasma NEFA, TG, glucose, and insulin levels were monitored at day 6 and day 13. At day 14, animals were sacrificed and tissue weights (mesenteric fat and liver) were measured. Liver TG contents were measured according to a method described previously.¹²⁾ In brief, liver was homogenized in methanol and extracted with chloroform. After evaporation of the solvent, the residue was redissolved in 2-propanol to measure TG contents by an enzymatic method.

Motor Nerve Conduction Velocity (MNCV) in STZ-induced Diabetic Mice Six-week-old ICR mice were used for the evaluation of DPN in type 1 diabetes model. Diabetes was induced with two injections of 120 mg/kg streptozocin (STZ) (Sigma-Aldrich; St. Louis, U.S.A.) dissolved in saline intraperitoneally after overnight fasting. Three weeks after STZ injection, 16 mice with plasma glucose level within $440\text{--}770 \text{ mg/dL}$ were selected and divided into two groups. The first group was fed normal diet (CRF-1) and the second group was fed 30 mg/kg/d JTP-96193 admixture diet for 8 weeks. Control mice were injected saline and were fed normal diet.

MNCV was measured after 8 weeks of treatment with JTP-96193 in accordance with our previous report.¹³⁾ Briefly, the sciatic nerve was stimulated at the sciatic notch and the Achilles tendon using adequate intensity under 37.5 mg/kg of sodium pentobarbital (Kanto chemical; Tokyo, Japan) and 3 mg/kg of diazepam (Maruishi Pharmaceutical; Osaka, Japan) anesthesia. Action potentials in the muscle were recorded *via* PowerLab (AD Instruments; Colorado Springs, CO, U.S.A.) through a needle electrode. MNCV was calculated from the delta latency between M-wave peaks divided by the distance of the nerve length measured.

Statistical Analysis All results except for enzyme assay were expressed as mean $\pm$ standard error of the mean (S.E.M.). Statistical analyses of differences between groups were performed using Dunnett's test or unpaired *t*-test after confirming normal distribution by *F* test or Bartlett test. Pearson's correlation coefficient was employed to evaluate correlations between TNF- α and NEFA levels in DIO mice. All statistical analyses were performed using Statlight 2000 (Yukms Corp., Tokyo, Japan) statistical software. Differences were accepted as significant at $p < 0.05$.

RESULTS

TACE Inhibitory Effect of JTP-96193 In a series of studies searching for drugs that potently and selectively inhibit TACE, we produced an optimal compound, JTP-96193 (Fig. 1). Specificity of JTP-96193 to TACE and related enzymes

was evaluated by enzyme assay. A summary of IC_{50} values to TACE and other enzymes for JTP-96193 is given in Table 1. IC_{50} of JTP-96193 to human TACE was 5.4 nM and JTP-96193 has more than 1800-fold selectivity against ADAM10 (has highest similarity of catalytic domain among ADAM family members), ADAMTS13 (close relative of the ADAM family), and matrix metalloproteinase (MMP)-14 (one of the factors responsible for musculoskeletal syndrome). Our preliminary study revealed that analogs of JTP-96193 have more than 500-fold selectivity against other MMP family members such as MMP-1, -2, -3, -7, -8, -9, -10, -12, -13, -15, -16, -17, and -24 (data not shown).

Next, TACE inhibitory effect of JTP-96193 was evaluated in rat whole blood assay. LPS-induced TNF- α production in rat whole blood was inhibited dose dependently by JTP-96193 pre-treatment from 0.1 μ M (Fig. 2A). JTP-96193 completely inhibited TNF- α production at 10 μ M, and the IC_{50} in *in vitro* whole blood assay was 0.17 μ M. To assess the effect of TACE inhibitor in *in vivo*, JTP-96193 was orally administered to Lewis rats and the plasma TNF- α concentration induced by LPS injection was measured. As a result, JTP-96193 significantly inhibited TNF- α production from dose of 1 mg/kg (Fig. 2B). The inhibitory effect of JTP-96193 on *in vivo* TNF- α production was dose-dependent and the ED_{50} was 3.8 mg/kg.

Epididymal Fat TNF- α and NEFA in DIO Mice Insulin resistance in adipose tissue is widely known as a key factor of diabetes. We evaluated the effect of JTP-96193 on epididymal fat tissue in DIO mice, a popular model of obesity and insulin resistance. Compared to control mice fed LFD, body weight of mice fed HFD was significantly heavier (data not shown). After 4 weeks of treatment, epididymal fat was stimulated with LPS to produce TNF- α and NEFA. Epididymal fat from HFD-fed mice produced significantly high concentra-

tion of TNF- α compared to fat from LFD-fed mice (Fig. 3A). JTP-96193 dose-dependently reduced the TNF- α release from fat tissue. Production of NEFA from epididymal fat was also increased in HFD-fed group, and JTP-96193 tended to inhibit NEFA production (Fig. 3B). Release of TNF- α and NEFA from epididymal fat was strongly correlated (Fig. 3C).

Blood Parameters in Diabetic KK-A^y Mice Anti-diabetic effect of JTP-96193 on type 2 diabetes model, KK-A^y mice, was evaluated. Body weight and food consumption were not different among the three groups (Table 2). Plasma NEFA level was reduced by both 3 and 30 mg/kg treatment with JTP-96193 through 2 weeks (Fig. 4A). Similarly, plasma TG levels were also decreased especially in 30 mg/kg JTP-96193-treated group (Table 2). In the tissue, mesenteric fat weight and liver TG content were tended to decrease by JTP-96193 treatment (Table 2). Plasma insulin and glucose levels were decreased only in 30 mg/kg JTP-96193-treated group (Figs. 4B, C).

Effect of TACE Inhibitor on DPN in STZ-Induced Diabetic Mice In STZ-induced diabetic mice, a type 1 diabetes animal model, body weight was decreased although food consumption was increased (Figs. 5A, B). Blood insulin level was decreased (Fig. 5C) and blood glucose level, measured as HbA1c, was obviously increased (Fig. 5D) as breakdown of pancreas developed in this animal model. These changes were not affected by 8 weeks of treatment with JTP-96193. Blood TACE activity was slightly increased in STZ mice and was tended to decrease by JTP-96193 treatment (Fig. 5E). Sciatic MNCV was significantly decreased and the treatment with JTP-96193 prevented this microvascular complication in STZ mice (Fig. 5F). Furthermore, urinary protein excretion tended to increase in diabetic animals and JTP-96193 slightly reduced proteinuria (control: 1.04 ± 0.65 mg/6h, STZ: 1.42 ± 0.54 mg/6h, STZ + JTP-96193: 0.99 ± 0.49 mg/6h). Creatinine clearance was significantly increased in diabetic mice

Fig. 1. Chemical Structure of JTP-96193

Fig. 2. TACE Inhibitory Activity of JTP-96193 in Rat Whole Blood

(A) TACE inhibitory activity of JTP-96193 in rat whole blood assay. LPS-induced TNF- α production in rat whole blood was inhibited by JTP-96193 pre-treatment (IC_{50} = 0.17 μ M). (B) The effect of JTP-96193 on LPS-induced TNF- α production in rat plasma in *in vivo* evaluation (ED_{50} = 3.8 mg/kg). Each value represents mean $\pm$ S.E.M. (n = 3). $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ vs. vehicle-treated group (Dunnett's test).

Table 1. Enzyme Inhibitory Activity on TACE and Selectivity of JTP-96193

Enzyme	IC_{50} (nM)	Selectivity
TACE (ADAM17)	5.4	—
ADAM10	>10000	>1850
MMP-14	>10000	>1850
ADAMTS-13	>10000	>1850

Fig. 3. The Effect of JTP-96193 on Adipose Tissue in DIO Mice

Epididymal fat was stimulated with LPS to produce TNF- α and NEFA after 4 weeks of treatment with JTP-96193. (A) Epididymal fat from HFD-fed mice produced significantly high concentration of TNF- α compared to fat from LFD-fed mice, and was reduced by treatment with JTP-96193. (B) NEFA production from epididymal fat was also increased in HFD-fed group, and was inhibited by JTP-96193. Each value represents mean $\pm$ S.E.M. ($n = 6-8$). * $p < 0.05$, ** $p < 0.01$ vs. LFD-fed group (unpaired t -test), †† $p < 0.01$ vs. vehicle-treated HFD-fed group (Dunnett's test). (C) Production of TNF- α and NEFA was strongly correlated (Pearson's correlation coefficient).

Fig. 4. Anti-diabetic Effect of JTP-96193 on KK-A y Mice

Plasma NEFA level (A) and insulin level (B) were significantly reduced by JTP-96193 treatment. Plasma glucose levels tended to decrease only at higher dose in JTP-96193-treated group (C). Each value represents mean $\pm$ S.E.M. ($n = 6-7$). † $p < 0.05$, †† $p < 0.01$ vs. control group (unpaired t -test).

Table 2. Body Weight, Food Consumption, and Lipid Parameters in KK-A y Mice

	Control	JTP-96193 (3 mg/kg)	JTP-96193 (30 mg/kg)
Body weight (g)	36.7 $\pm$ 0.6	37.2 $\pm$ 0.9	36.1 $\pm$ 0.6
Cumulative food consumption (g/14d)	72.1 $\pm$ 2.5	75.9 $\pm$ 3.0	72.4 $\pm$ 2.2
Plasma TG (mg/dL)	730.2 $\pm$ 55.5	680.5 $\pm$ 63.0	462.1 $\pm$ 66.6 [†]
Mesenteric fat weight (g)	0.87 $\pm$ 0.04	0.80 $\pm$ 0.05	0.78 $\pm$ 0.08
Liver weight (g)	2.25 $\pm$ 0.04	2.25 $\pm$ 0.07	2.15 $\pm$ 0.09
Liver TG content (mg/g tissue)	27.7 $\pm$ 2.6	23.9 $\pm$ 2.0	21.0 $\pm$ 3.3

Each value is represented as mean $\pm$ S.E.M. ($n = 6-8$). † $p < 0.05$ vs. control group (Dunnett's test).

and tended to decrease by JTP-96193 treatment (control: 0.17 ± 0.04 mL/min, STZ: 0.25 ± 0.08 mL/min [$p < 0.05$ vs. control], STZ + JTP-96193: 0.20 ± 0.07 mL/min).

DISCUSSION

A number of orally bioavailable TNF- α inhibitors have been developed since the great achievement of anti-TNF- α biological drugs. Inhibition of pro-TNF- α processing to the active TNF- α form by TACE inhibitor is one of the options to control RA.^{14,15} However, the musculoskeletal side effects are caused due to simultaneous inhibition of other MMPs, because of similarity of binding subsites between TACE and

other MMPs. Therefore, it is desired that the selective TACE inhibitors avoid these side effects caused by inhibition of other MMPs. In the present study, we synthesized a novel TACE inhibitor, JTP-96193, which potently ($IC_{50} = 5.4$ nM) and selectively (more than 1800-fold higher than other MMPs) inhibits TACE enzyme activity. A great deal of effort has been devoted by pharmaceutical companies to develop a number of TACE inhibitors. BMS-561392 or DPC-333 (Bristol-Myers Squibb Company) inhibits TACE activity with IC_{50} value of 0.20 nM in *in vitro* study and its selectivity on TACE activity is more than 100-fold over other MMPs.¹⁶ Apratastat (TMI-005; Wyeth Pharmaceuticals) shows TACE inhibitory activity with IC_{50} value of 440 nM in *in vitro*.¹⁷ Although both drugs show good *in vitro* profiles, clinical phase II trials had failed due to hepatotoxicity and lack of efficacy, respectively.¹⁸ Previous TACE inhibitors, including these two compounds, have some disadvantages because their chemical structures contain hydroxamate. Low bioavailability and toxic metabolites are common problem of this class of compounds. To overcome these disadvantages of hydroxamic acid group, we developed a non-hydroxamate, thiadiazolone-derivative TACE inhibitor.

Because we have not performed toxicological study of JTP-96193 yet, the safety findings are limited to those accompanied pharmacological studies. However, from a standpoint of TACE inhibition, Horiuchi *et al.* reported that the TACE deficient mice displayed no evident of pathological phenotypes or histopathological defects.¹⁹ Considering from the aspect of compound itself, we have preliminary data of analogs of

Fig. 5. Effects of JTP-96193 on STZ-Induced Diabetic Mice

STZ-induced diabetic mice showed decreased body weight (A) and increased food consumption (B). Plasma insulin was almost diminished (C) and plasma glucose level (evaluated as HbA1c level) was obviously increased correspondingly to the change of plasma insulin level (D). All these changes were not affected by treatment with JTP-96193 (A–D). Blood TACE activity was slightly increased in STZ mice and tended to decrease by JTP-96193 treatment (E). Sciatic MNCV was significantly decreased and treatment with JTP-96193 prevented this microvascular complication. Each value represents mean $\pm$ S.E.M. ($n = 6-7$). * $p < 0.05$, ** $p < 0.01$ vs. control mice, †† $p < 0.01$ vs. vehicle-treated STZ mice (unpaired t -test).

JTP-96193, the previously developed TACE inhibitors with non-hydroxamate structure. These analogs showed negative or less pathological changes in the liver (necrosis, microgranuloma, and infiltration of inflammatory cells) after 10d administration (300mg/kg) while the histopathological findings were obvious in BMS-561392 (hydroxamate structure) treated mice at same dose. According to their chemical structure, hydroxamate contain compounds such as BMS-561392 and apratastat are metabolized to cytotoxic hydroxylamine.²⁰ Thiadiazolone-derived TACE inhibitors are our original compounds without containing hydroxamate and therefore this class of compounds does not produce hydroxamine. Although it is not known whether thiadiazolone-derived compound generally shows good bioavailability, area under the blood concentration–time curve (AUC) and biological half-life ($t_{1/2}$) of JTP-96193 after 10mg/kg oral administration to rat is 12500nM·h and 4.0h, respectively and those of BMS-561392 are 790nM·h and 0.8h, respectively. Thus, we are considering that JTP-96193 is safer and more bioavailable TACE inhibitor compared to hydroxamate-derived compounds and that the risk of systemic inhibition of TACE by JTP-96193 is minimal.

TACE inhibitors have been developed to treat autoimmune diseases with inflammation (e.g., RA) as TNF- α inhibitors. In addition, chronic inflammation is also deeply associated with obesity²¹ and excess expression of TNF- α in adipose tissue causes peripheral insulin resistance in obese animal and humans.^{22,23} Several reports support the relationship between TACE and insulin resistance. TACE^{+/-} mice fed HFD, a model of loss of TACE function, show tolerance to obesity and insulin resistance.²⁴ Experiments with Timp3 (tissue inhibitor of metalloproteinase 3; an intrinsic inhibitor of MMPs, including TACE) knockout mice, a model of TACE gain of function, exhibit glucose intolerance and insulin resistance.²⁵ Furthermore, some single-nucleotide polymorphisms (SNPs) of

TACE/ADAM17 significantly associate with insulin-resistance phenotypes and obesity risk.²⁶ Therefore, in the present study, we evaluated the pharmacological properties of JTP-96193 on obesity, insulin resistance, and diabetes. JTP-96193 decreased TNF- α production in adipose tissue in DIO mice and prevented development of diabetes and improved insulin resistance in KK-A^j mice. Both TNF- α and NEFA are known to develop insulin resistance. Therefore it seems that plasma NEFA level was slightly changed by inhibiting TNF- α production, but insulin resistance was much improved by reduction of both TNF- α and NEFA (Fig. 4).

In 2011, Yamakawa *et al.* reported that inactivation of TNF- α ameliorates DPN in TNF- α -deficient mice and infliximab-treated mice.⁹ In their study, treatment with infliximab improved NCV and tail flick test score. Therefore, we hypothesized that TACE inhibitor also improves DPN. Although we could evaluate neither tail flick test nor nerve fiber density in mice, JTP-96193 directly prevented development of DPN in STZ-induced diabetic mice without any effect on blood glucose or insulin level. Therefore, TACE inhibitor may have been worked as neuroprotective both in functionally and in histopathologically. It is reported that BMS-561392 shows its pharmacological effect on cultured dorsal root ganglion (DRG).²⁷ Thus, the neuroprotective effect of JTP-96193 is considered due to inhibition of TNF- α production in DRG.

Similarly, increased urinary protein excretion tended to decrease by treatment with JTP-96193, consistent with the previous report treating with infliximab.¹⁰ It has been reported that angiotensin II (Ang II) causes kidney damage by inducing systemic/glomerular hypertension and renal overgrowth.²⁸ Because Ang II-induced renal lesion are reduced in TGF- α (also shed by TACE) deficient mice or mice treated TACE inhibitor,²⁹ the widespread function of TACE as sheddase may deeply involve in chronic kidney disease. Although infliximab

prevents urinary albumin excretion in STZ-induced diabetic rats,¹⁰⁾ urinary protein excretion was not significantly decreased by JTP-96193 treatment. The difference may be due to parameters; albumin excretion is more sensitive than protein excretion, generally. In addition, creatinine clearance was also tended to decrease by treatment of JTP-96193. Taking these things together, it seems that JTP-96193 has renoprotective potential; however, because of renal toxicity induced by STZ treatment,³⁰⁾ further study is needed to evaluate renoprotective effect of JTP-96193. Since all currently available TNF- α inhibitors is molecular targeted drug, it is unrealistic to treat these inhibitors for diabetes. Although the renoprotective effect was not significant in this study, JTP-96193 is orally administrable drug that improve insulin resistance and DPN which is a desirable profile to treat diabetes; thus, this compound is expected to be clinically applicable.

More recently, TACE inhibitor has also been developed as an anti-tumor drug, with its inhibitory effect on epidermal growth factor receptor (EGFR) signaling in cancer. Aderbasib (INCB-7839; Incyte Corporation), a dual inhibitor of ADAM 10 and 17, is currently in phase I/II clinical trials with rituximab, a chimeric anti-human CD20 antibody, for treatment of diffuse large B cell non-Hodgkin lymphoma (NCT02141451). Considering that more than 80 substrates related to cancer or inflammation are regulated by TACE, the future development of TACE inhibitors as multi-functional drugs for diseases in addition to RA and diabetes is encouraged.

In conclusion, effects of novel potent and selective TACE inhibitor JTP-96193 on DPN in addition to effects on insulin resistance have been shown in the present study. Widespread additive pharmacological properties of TACE inhibitors will become a new treatment option of type 2 diabetes accompanied by its microvascular complications.

Conflict of Interest Mariko Maekawa, Hironobu Tadaki, Daisuke Tomimoto, Chihiro Okuma, Ryuhei Sano, Yukihiro Ishii, Yoshiaki Katsuda, Hiromi Yoshiuchi, Reina Kakefuda and Tomohiko Sasase are employees of Japan Tobacco Inc. Takeshi Ohta has no conflict of interest.

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