Anti-inflammatory role of DPP-4 inhibitors in a nondiabetic model of glomerular injury

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Higashijima Y, Tanaka T, Yamaguchi J, Tanaka S, Nangaku M. Anti-inflammatory role of DPP-4 inhibitors in a nondiabetic model of glomerular injury. Am J Physiol Renal Physiol 308: F878–F887, 2015.—Dipeptidyl peptidase (DPP)-4 is an enzyme that cleaves and inactivates incretin hormones capable of stimulating insulin secretion from pancreatic β-cells. DPP-4 inhibitors are now widely used for the treatment of type 2 diabetes. Experimental studies have suggested a renoprotective role of DPP-4 inhibitors in various models of diabetic kidney disease, which may be independent of lowering blood glucose levels. In the present study, we examined the effect of DPP-4 inhibitor, anagliptin (300 mg·kg⁻¹·day⁻¹ mixed with food) and a glucagon-like peptide-1 receptor agonist, exendin-4 (10 mg/kg sc), similarly reduced CD68-positive macrophages infiltration in the kidney, which was associated with a nonsignificant tendency to ameliorate glomerular injury and reduce proteinuria. Another DPP-4 inhibitor, alogliptin (20 mg·kg⁻¹·day⁻¹) or vehicle for 7 days orally by gavage. Alogliptin significantly reduced the number of CD68-positive inflammatory macrophages in the kidney, which was associated with a nonsignificant tendency to ameliorate glomerular injury and reduce proteinuria. Importantly, GLP-1R expression is not only limited to pancreatic β cells but is also observed in multiple organs, such as the gut, lungs, heart, kidney, and central nervous system (32). This widespread distribution of GLP-1R has raised our expectations regarding its functionality, particularly its protective roles in extrapancreatic tissue, which could be mediated by GLP-1R agonists and DPP-4 inhibitors currently available. The protective effects of GLP-1R agonists and DPP-4 inhibitors have been well documented in diabetic kidney disease (DKD) models (24, 36). However, only a few studies have examined the effects of GLP-1R agonists and DPP-4 inhibitors in non-DKD models, such as on cisplatin-induced nephrotoxicity, ischemia-reperfusion injury (6, 16), and the remnant kidney (14).

In the present study, we investigated the potential role of DPP-4 inhibitors in the rat Thy-1 glomerulonephritis model. DPP-4 inhibitors reduced macrophage infiltration to the injured kidney, glomerular injury, and proteinuria. The number of classically activated, inflammatory, M1-like macrophages was reduced, whereas that of alternatively activated, tissue repair-M2-like macrophages was not affected by the treatment with DPP-4 inhibitors. These findings suggest that the protective effect of DPP-4 inhibitors was mainly mediated by its anti-inflammatory action.

MATERIALS AND METHODS

Animals. All animal experiments were conducted in accordance with guidelines of the Committee on Ethical Animal Care and Use of the University of Tokyo (Approval no. P14-025 and P14-041). Male Sprague-Dawley rats aged 6 wk and male C57BL6/J mice aged 9 wk were purchased from CLEA Japan (Shizuoka, Japan). Animals were housed in individual cages in a temperature- and light-controlled environment and had ad libitum access to chow and water.

Drug administration. Anti-Thy-1 glomerulonephritis was induced in rats by a single intravenous injection of IgG (OX-7) mouse monoclonal anti-Thy-1.1 antibody (1.2 mg/kg). DPP-4 inhibitors, alogliptin and anagliptin, were generously provided by Takeda Pharmaceutical, (Osaka, Japan) and Sanwa Kagaku Kenkyusho (Aichi, Japan), respectively. In the first experiment, rats were treated with alogliptin (10 mg/kg, twice a day) or vehicle orally by gavage for 7 days after Thy-1 nephritis induction. Rats were divided into four groups (control: n = 9, control alogliptin: n = 9, Thy-1: n = 14, and Thy-1 alogliptin: n = 15). In the second experiment, rats were treated with anagliptin (300 mg·kg⁻¹·day⁻¹ mixed with food) or vehicle and were divided into four groups (control: n = 3, control anagliptin: n = 3, Thy-1: n = 5, and Thy-1 anagliptin: n = 5). In the third experiment, rats were treated with the GLP-1R agonist exendin-4 (5 μg/kg, twice a day. AstraZeneca, Osaka, Japan) via subcutaneous injection for 7 days and were divided into four groups (control: n = 4, control exendin-4: n = 4, Thy-1: n = 6, and Thy-1 exendin-4: n = 6). Rats were housed in metabolic cages for overnight collection of urine from days 6 to 7 and then euthanized for tissue analysis.

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Renal histological analyses. Tissues were fixed in formalin and embedded in paraffin. Three-micrometer sections were stained with periodic acid-Schiff reagent and counterstained with hematoxylin. Quantification of renal histology was performed as previously described (3). Briefly, 30 glomeruli/section were scored using the following system: 0 = normal appearance, 1 = mesangial expansion and/or hypercellularity, and 2 = microaneurysms, necrosis, capsular herniations, or matrix or cellular crescents. Quantitative analyses were performed in a blinded manner.

Immunohistochemistry. Formalin-fixed or methyl-Carnoy-fixed and paraffin-embedded tissues were sectioned at 3 μm for immunohistochemistry. The following primary antibodies were used: polyclonal goat anti-SOD1 (Santa Cruz Biotechnology, Santa Cruz, CA), polyclonal rabbit anti-nitrotyrosine (Sigma-Aldrich, St. Louis, MO), monoclonal mouse anti-CD68 (Merck Millipore, Billerica, MA), monoclonal mouse anti-CD163 (AbD Serotec, Kidlington, UK), and monoclonal rabbit anti-CD206 (Abcam, Cambridge, UK). As a secondary antibody, biotinylated horse anti-mouse IgG (Vector Laboratories, Burlingame, CA), goat anti-rabbit IgG antibody (Vector Laboratories), or biotinylated rabbit anti-goat IgG antibody (Dako Japan, Tokyo, Japan) was used as appropriate. Horseradish peroxidase-labeled avidin D (Vector Laboratories) was used as a third antibody, and the reaction product was visualized by treatment with 3,3′-diaminobenzidine tetrahydrochloride. CD68- and CD163-positive cells were counted in five randomly selected cortical fields at ×100 magnification.

Measurement of DPP-4 activity and its substrates. DPP-4 activity in plasma and homogenates of the renal cortex was measured using an assay kit (Enzo Life Sciences, Farmingdale, NY). Renal DPP-4 activity was normalized according to the weight of the homogenized tissue sample. Active GLP-1 levels in plasma were measured using an enzyme immunoassay kit (Merck Millipore).

Real-time PCR. RNA was isolated using RNAiso Plus (Takara, Shiga, Japan) and reverse transcribed with PrimeScript RT Master Mix (Perfect Real Time, Takara). One-twentieth (vol/vol) of the synthesized cDNA was used as a template for PCR quantification. PCR was performed on CFX96 (Bio-Rad, Hercules, CA) with the THUNDERBIRD SYBR qPCR mix (Toyobo, Osaka, Japan). Relative expression levels were calculated using β-actin mRNA as a reference. Primers for quantification are shown in Table 1.

Cell preparation and RT-PCR. RAW264 was purchased from Seiyaku (Tokara, Shiga, Japan) and reverse transcribed with PrimeScript RT Master Mix (Perfect Real Time, Takara). One-twentieth (vol/vol) of the synthesized cDNA was used as a template for PCR quantification. PCR was performed on CFX96 (Bio-Rad, Hercules, CA) with the THUNDERBIRD SYBR qPCR mix (Toyobo, Osaka, Japan) or KAPA SYBR Fast Universal 2× qPCR Master Mix (Kapa Biosystems, Wilmington, MA). Relative expression levels were calculated using β-actin mRNA as a reference. Primers for quantification are shown in Table 1.

Effect of DPP-4 inhibitors on glomerular injury and proteinuria in the rat Thy-1 nephritis model. DPP-4 inhibitors are antidiabetic drugs acting on incretins, which exert glucose-lowering effects only in hyperglycemia. Therefore, they are not likely to induce hypoglycemia in young, nondiabetic animals. In a pilot study, we confirmed that alogliptin did not affect the fasting blood glucose level (control: 79.8 ± 4.1 mg/dl, control alogliptin: 73.4 ± 3.3 mg/dl, Thy-1: 74.2 ± 2.9 mg/dl, and Thy-1 alogliptin: 74.5 ± 1.9 mg/dl) or systolic blood pressure (control: 120.7 ± 6.7 mmHg, control alogliptin: 122.7 ± 10.5 mmHg, Thy-1: 116.4 ± 2.4 mmHg, and Thy-1 alogliptin: 119.3 ± 1.3 mmHg).

The rat Thy-1 nephritis model is characterized by early mesangiolysis (by days 2–3) followed by subsequent mesangial cell proliferation, matrix expansion, and crescent formation, which peaks on day 7. To see the potential effects of alogliptin in the early phase in this model, we performed pathological analysis, but reduction of mesangiolysis was not

Table 1. List of primers for real-time PCR

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>Species</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD68</td>
<td>Rat</td>
<td>5'-TGGTGGTGAATGCTGTTAGGCG-3'</td>
<td>5'-GTGGAGCGCTTTTGTGAGG-3'</td>
</tr>
<tr>
<td>CD163</td>
<td>Rat</td>
<td>5'-CCAAGAGGGTTAGTTATCGCTC-3'</td>
<td>5'-TACGTTGCCGGTGCGTTAAGG-3'</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Rat</td>
<td>5'-GACAGAGGACCCGACAGACAGAC-3'</td>
<td>5'-CAACAGGCCGACAGACAGAC-3'</td>
</tr>
<tr>
<td>RANTES</td>
<td>Rat</td>
<td>5'-CCTCGACTCCCATGCTCGTAC-3'</td>
<td>5'-GGTTGCAATGTCGCCCTC-3'</td>
</tr>
<tr>
<td>CCR2</td>
<td>Rat</td>
<td>5'-GTTGAGACAAAGCTGCTGCCG-3'</td>
<td>5'-ATGTTGAGCTCTGCTGCG-3'</td>
</tr>
<tr>
<td>CCR5</td>
<td>Rat</td>
<td>5'- AACCTTGCGCTTTTGTGAGG-3'</td>
<td>5'-GAGATGGCGACAGTGAAGC-3'</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Rat</td>
<td>5'-GACCTTCAAGAACAGAGAAGAC-3'</td>
<td>5'-GAGTTCTGGAAGTTCACCTC-3'</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Rat</td>
<td>5'-GAGAGAGAATAAGTGTGCTCT-3'</td>
<td>5'-TTACATAGGACAGTTGCTC-3'</td>
</tr>
<tr>
<td>IL-6</td>
<td>Rat</td>
<td>5'-GGCTTCAAACGTGAGAGG-3'</td>
<td>5'-GAGATGGCGACAGTGAAGC-3'</td>
</tr>
<tr>
<td>β-Actin</td>
<td>Rat</td>
<td>5'-GTTTCTTCAATGAATGTCGCTTG-3'</td>
<td>5'-TCCGTTGCAACAATCTTCTT-3'</td>
</tr>
</tbody>
</table>

MCP-1, monocyte chemotactic protein-1; RANTES, regulated on activation, normal T cell expressed and secreted; CCR, chemokine (C-C motif) receptor.
observed by alogliptin treatment at day 3 (data not shown). Thus, we next evaluated glomerular pathology on day 7. Alogliptin treatment nonsignificantly reduced the total injury score of glomeruli (Thy-1: 35.1 ± 1.3 and Thy-1 alogliptin: 31.4 ± 1.7, \( P = 0.37 \); Fig. 1, A–G). The number of glomeruli categorized as score 0 (no injury) was slightly increased from 1.9 ± 1.1 to 7.6 ± 2.9 and that belonging to score 2 (severe injury) was moderately decreased from 19.1 ± 3.7 to 12.4 ± 3.5, although the results were not significant (Fig. 1H). Consistent with pathological analyses, a nonsignificant trend toward a reduction in proteinuria was observed (Fig. 1K). Serum creatinine levels were not altered in this model (control: 0.33 ± 0.03 mg/dl, control alogliptin: 0.30 ± 0.01 mg/dl, Thy-1: 0.36 ± 0.02 mg/dl, and Thy-1 alogliptin: 0.34 ± 0.07 mg/dl).

**Equivocal impact of the DPP-4 inhibitor on oxidative stress.** Previous studies (8, 22, 25) have reported that DPP-4 inhibitors exhibit renoprotective effects through antioxidative stress in experimental DKD models. Thus, we examined the effect of alogliptin on oxidative stress by immunohistological analyses. Expression levels of the antioxidant enzyme SOD1 in the renal cortex were decreased by disease induction but were not affected by alogliptin (Fig. 2, A–E). In addition, an oxidative stress marker, nitrotyrosine, was not detected in this model (Fig. 2, F–J), making it less likely that the antioxidant property was the major determinant of injury.

**DPP-4 inhibitor reduces macrophage infiltration in the rat Thy-1 model.** Conversely, a reduction in albuminuria and glomerular mesangial matrix expansion by DPP-4 inhibition have been reported in streptozotocin (STZ)-induced type 1 diabetes, which was attributed to anti-inflammatory action via GLP-1 signaling (18). We followed this model and then examined the possible effects of DPP-4 inhibitors on inflammation. Immunohistochemical analysis demonstrated that alogliptin significantly reduced the number of CD68-positive macrophages in the tubulointerstitial area (Fig. 3, A–E). Another DPP-4 inhibitor, anagliptin, similarly, albeit nonsignificantly (\( P = 0.77 \)), reduced the number of CD68-positive cells (control: 37.3 ± 4.9, control anagliptin: 31.9 ± 1.6, Thy-1: 154.4 ± 31.3, and Thy-1 anagliptin: 120.6 ± 28.8). Nevertheless, the results of two independent DPP-4 inhibitors suggest that the suppression of infiltrating macrophages is a class effect. Next, we focused on subsets of macrophages generically known as M1 and M2 macrophages. Because we were unable to reproduce the stain of CD169-positive macrophages by immunohistochemistry, we evaluated the number of CD163-positive macrophages infiltrating the kidney, which did not change (Fig. 3, F–J).
Similar results were observed in CD206-positive macrophages (data not shown). This indicated that the M2 subset was not significantly altered by alogliptin. Several inflammatory mediators, such as chemokines and cytokines, are known to play crucial roles in macrophage infiltration. We thus analyzed mRNA expression of representative inflammatory mediators in the renal cortex using quantitative RT-PCR. Treatment with alogliptin did not affect mRNA levels of MCP-1 and regulated on activation, normal T cell expressed and secreted (RANTES) but showed a nonsignificant trend toward decreases in chemokine (C-C motif) receptor (CCR)2, CCR5, IL-1β, and IL-6 (Fig. 3, K–S).

Plasma and renal DPP-4 activity in the rat Thy-1 model. In an effort to elucidate the mechanisms underlying these observations, we examined DPP-4 activity in plasma and kidney homogenates and found that it was significantly reduced after the induction of Thy-1 nephritis. Alogliptin further significantly reduced both plasma and renal DPP-4 activity, as expected (Fig. 4, A and B). Consistently, plasma GLP-1 levels were significantly increased again by treatment with alogliptin (Fig. 4C).

Reduction in infiltrating macrophages by the GLP-1R agonist. GLP-1 is the most clearly established substrate of DPP-4 in vivo, and most of the protective effects of DPP-4

Fig. 2. Effects of alogliptin on renal oxidative stress. Oxidative stress was estimated by the immunohistochemistry of SOD1 (A–E) and nitrotyrosine (F–J). A–J: representative staining of control (A and F), control alogliptin (B and G), Thy-1 (C and H), Thy-1 alogliptin (D and I) groups as well as unilateral obstruction (used as the positive control; E and J). Bars = 100 μm (magnification: ×200).

A–J.

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inhibitors are phenocopied by GLP-1R agonists in DKD models. Thus, we reasoned that GLP-1R signaling might be responsible for the suppression of macrophage infiltration and used exendin-4 to examine the effects of GLP-1R agonist in the rat Thy-1 model. Although exendin-4 did not reduce glomerular injury (Thy-1: 35.4 ± 0.7 and Thy-1 exendin-4: 37.2 ± 1.5) and proteinuria (Thy-1: 29.0 ± 3.4 mg/12 h and Thy-1 exendin-4: 38.4 ± 6.4 mg/12 h), the number of CD68-positive macrophages infiltrating the kidney was significantly reduced by exendin-4 treatment (Fig. 5, A–E), with a parallel increase in the CD163-positive subpopulation (Fig. 5, F–J). Treatment with exendin-4 reduced mRNA expression of TNF-α in the control Thy-1 alogliptin ++--

Fig. 3. Effects of alogliptin on renal inflammation. Renal macrophage infiltration was assessed by the immunohistochemistry of macrophage markers CD68 and CD163. A–D and F–I: representative images of CD68 (A–D) and CD163 (F–I) stains. Bars = 100 μm (magnification: ×200). E and J: CD68-positive cells (E) and CD163-positive cells (J) were counted and expressed as counts per field. K–S: mRNA expression of inflammation-related genes in the renal cortex was quantified by real-time PCR. MCP-1, monocyte chemotactic protein-1; RANTES, regulated on activation, normal T cell expressed and secreted; CCR, chemokine (C-C motif) receptor. Data are expressed as means ± SE. *P < 0.05 compared with the control group; †P < 0.05 compared with the Thy-1 group.
kidney, and this decrease was associated with nonsignificant decreases in RANTES, CCR2, CCR5, IL-1β, and IL-6 mRNA expressions (Fig. 5, K–S).

GLP-1R agonist, but not the DPP-4 inhibitor, reduces MCP-1-stimulated macrophage infiltration in vitro. Finally, ex vivo transmigration assays were performed to examine the direct effect of DPP-4 inhibitor on macrophages. To address this, we checked mRNA expression of GLP-1R and DPP-4 in macrophage cells. Although both GLP-1R and DPP-4 mRNAs were readily detectable in peritoneal macrophages, they were not detectable in a RAW264 cell line, within the amplification range (Fig. 6, A and H). Therefore, we performed migration assays using peritoneal macrophages in subsequent experiments. Exendin-4 dose dependently reduced MCP-1-stimulated macrophage infiltration to the lower chamber (Fig. 6, B–G). Conversely, alogliptin did not significantly reduce macrophage infiltration (Fig. 6, I–N), raising the possibility that the observed suppression of macrophage infiltration in vivo was mediated via GLP-1-dependent signaling subsequent to DPP-4 inhibition.

**DISCUSSION**

Therapeutic effects of GLP-1R agonists and DPP-4 inhibitors are currently being evaluated in DKD models. For example, previous studies (8, 17, 18, 22, 25) have shown that the GLP-1R agonist and DPP-4 inhibitor ameliorated STZ-induced DKD. In models of type 2 diabetes, GLP-1R agonists also ameliorated kidney injury (5, 27). In contrast, only a few studies have examined the effects of GLP-1R agonists and DPP-4 inhibitors in non-DKD models. In acute kidney injury models, GLP-1R agonist and DPP-4 inhibitor ameliorated cisplatin-induced nephrotoxicity and ischemia-reperfusion injury (6, 16). In a chronic kidney disease model, the DPP-4 inhibitor attenuated renal dysfunction and structural damage in the remnant kidney (14).

Shinokita et al. (30) previously reported that monoclonal antibody against DPP-4 considerably reduced proteinuria and mesangial expansion via the suppression of the complement cascade in the rat Thy-1 model. Contrary to this report, we observed a nonsignificant trend toward reductions in glomerular injury and proteinuria in the present study. DPP-4 (CD26), on the one hand, is an important molecule that functions as a cleaving enzyme and also serves as a surface receptor and/or costimulatory protein, especially in the immune response. For example, DPP-4/CD26 is a T cell activation antigen, but its enzymatic activity is not required for the signaling function in T cells (7). In addition, the expression of DPP-4/CD26, but not DPP-4 activity, is associated with the susceptibility of human immunodeficiency virus type 1 to CD4 T lymphocytes (26). In the rat Thy-1 model, T helper 1 cytokines produced by CD4 T lymphocytes contribute to the development of injury (12), and the suppression of cytokine production could be a valid target for therapy (11). Taken together, the results of the present study indicate that the expression of DPP-4, but not enzymatic activity, might be important for immune responses in CD4 T lymphocytes. Thus, the inhibition of DPP-4 activity only resulted in a nonsignificant reduction of glomerular injury and proteinuria in this model.

We focused on the anti-inflammatory effects of DPP-4 inhibitors. Two types of DPP-4 inhibitors, alogliptin and alogliptin, reduced macrophage infiltration to the injured kidney. This effect was associated with increased plasma GLP-1 levels. In addition, the GLP-1R agonist exendin-4 similarly reduced macrophage infiltration to the kidney. mRNA levels of chemokines such as MCP-1 and RANTES in the renal cortex were not affected by the treatments with alogliptin and exendin-4. Furthermore, additional ex vivo transmigration assays revealed that exendin-4, but not alogliptin, dose dependently reduced MCP-1-stimulated macrophage infiltration. These data indicate that DPP-4 inhibitors suppress macrophage infiltration via GLP-1-dependent signaling in the rat Thy-1 model.

Macrophages play an important role in the disease progression of glomerulonephritis (29). For example, MCP-1-neutralizing antibodies reduce macrophage infiltration and thereby ameliorate glomerular injury and proteinuria in a nephrototoxic serum glomerular nephritis model (23). In a lupus nephritis model, MCP-1 knockout MRL-Fas (lpr) mice exhibit less glomerular injury and reduced proteinuria, accompanied by a reduction in macrophage infiltration (37). In the Thy-1 model, Rampino et al. (28) reported that neutralization of macrophage-stimulating proteins reduces macrophage infiltration and leads to an attenuation of renal histology and proteinuria. In our study, treatment with alogliptin resulted in a nonsignificant trend toward reductions in glomerular injury and proteinuria. These trends were also associated with reduced macrophage infiltration, and, thus, these results were consistent with those

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### Fig. 4. Plasma and renal DPP-4 activity and plasma glucagon-like peptide (GLP)-1 levels.

- **Panel A**: Plasma DPP4 activity
  - Control
  - Thy-1
  - alogliptin

- **Panel B**: Renal DPP4 activity
  - Control
  - Thy-1
  - alogliptin

- **Panel C**: Plasma GLP-1 concentration
  - Control
  - Thy-1
  - alogliptin

Data are expressed as means ± SE. *P < 0.05 compared with the control group; †P < 0.05 compared with the Thy-1 group.
of Rampino et al. However, detailed molecular mechanisms involving DPP4 inhibitor suppression of macrophage infiltration merit further investigation.

Currently, macrophages can be broadly divided into classically activated (M1-like) or alternatively activated (M2-like) macrophages (31). However, whether M2-like macrophages contribute to the progression of kidney diseases remains controversial. In an ischemia-reperfusion injury model, M2-like macrophages promote tubular cell proliferation and tissue repair (20, 40). In contrast, in a polycystic kidney disease

Fig. 5. Effects of a GLP-1 receptor (GLP-1R) agonist, exendin-4, on renal macrophage infiltration. A–D and F–I: representative images of CD68 (A–D) and CD163 (F–I) stain. Bars = 100 μm (magnification: ×200). E and J: numbers of CD68-positive cells (E) and CD163-positive cells (J) expressed as counts per field. K–S: renal mRNA expression of inflammation-related genes was quantified by real-time PCR. Data are expressed as means ± SE. *P < 0.05 compared with the control group; †P < 0.05 compared with the Thy-1 group.
model, M2-like macrophages promote cyst cell proliferation, cyst growth, and fibrosis (33). Furthermore, in the Thy-1 model, prednisolone increased CD163 (M2-like) macrophages and exacerbated global glomerulosclerosis, whereas mizoribine reduced CD163-positive macrophages and alleviated mesangial expansion and glomerulosclerosis (13). In addition, epoetin/epoetin pegol (continuous erythropoietin receptor activator) ameliorated glomerular injury in the Thy-1 model, with a reduction in the number of CD163-positive macrophages (1). These reports suggest that M2-like macrophages contribute to the progression of glomerular injury in the Thy-1 nephritis model. In our study, DPP-4 inhibitors, but not the GLP-1R agonist, exhibited the tendency of reducing proteinuria, whereas the number of CD163-positive macrophages was not affected by treatment with the DPP-4 inhibitor, but it increased by treatment with the GLP-1R agonist. We speculate that the polarity in changes of macrophages require potent signaling of GLP-1R and that the increased number of M2-like macrophages by effects of the GLP-1R agonist might have negated the renoprotective effects observed with the DPP-4 inhibitor.

Contrary to many published papers, reducing renal macrophage infiltration did not result in a significant reduction in glomerular injury and proteinuria in our study. Alogliptin was administered to rats in reference to a previous study (19) in which the pharmacokinetics of alogliptin were closely examined and plasma DPP-4 inhibition in rats was observed through 12 h but not 24 h after a single dose of 10 mg/kg alogliptin. To achieve constant inhibition of plasma DPP-4, we administered 10 mg/kg alogliptin twice a day, and significant plasma DPP-4 increases were confirmed but failed to reduce proteinuria significantly. As to exendin-4, we used the same dose as described in a previous study (17) from another group (10 µg·kg⁻¹·day⁻¹) in which exendin-4 significantly reduced albuminuria in a STZ-induced type 1 diabetes model. In an additional experiment, rats in the Thy-1 group were treated with exendin-4 (5 µg/kg twice a day) from day –7 to
day 7, but proteinuria was not improved (data not shown). Based on this, we believe that the absent reduction in proteinuria was not due to insufficient DPP4 inhibition and GLP-1R stimulation but rather because of the robust degree of renal injury in which a reduction in macrophage infiltration alone was not sufficient to influence the disease course.

Takase et al. (35) used eicosapentaenoic acid (EPA) in the same model. Treatment with EPA significantly reduced CD68-positive macrophage infiltration into the tubulointerstitial area, which was associated with the prevention of tubulointerstitial injury. However, EPA did not reduce CD68-positive macrophage infiltration into glomeruli and failed to reduce proteinuria, suggesting that inhibition of macrophage infiltration in the glomerulus is important for reducing proteinuria and glomerular injury in the rat Thy-1 model. Indeed, many studies (3, 28) have shown that reductions of proteinuria and glomerular injury are related to the reduction of macrophages in glomeruli. In our study, the number of CD68-positive macrophages in glomeruli was not decreased by treatment with alogliptin (data not shown). Thus, this may be another reason why the reduction of macrophage infiltration did not result in reductions in glomerular injury and proteinuria.

Apart from GLP-1, DPP-4 can cleave multiple substrates, such as brain-derived natriuretic peptide, substance P, neuropeptide Y, peptide YY, high-mobility group protein B1, and stromal-derived factor-1 (SDF-1). Among these, the protective effects of SDF-1 have been extensively examined. In a model of myocardial infarction, DPP-4 inhibition reduced infarct size and improved cardiac function via myocardial homing of circulating stem cells (9, 39). The DPP-4 inhibitor also recruits regenerated stem cells via SDF-1α signaling and improves lung ischemia-reperfusion injury (15). Conversely, Katagiri et al. (16) reported that the DPP-4 inhibitor ameliorated cisplatin-induced renal injury via GLP-1 but not SDF-1α signaling. Thus, multiple targets of DPP-4 appear to play a role in the protection against acute kidney injury, and substrates other than GLP-1 might have contributed to the tendency of proteinuria reduction in our study.

In conclusion, DPP-4 inhibitors reduce macrophage infiltration directly via the GLP-1-dependent pathway and have a trend to ameliorate proteinuria in the rat Thy-1 nephritis model. Control of inflammation by DPP-4 inhibitors might have the potential to ameliorate the progression of non-DKDs. Currently, studies on the renoprotective effects of DPP-4 inhibitors in such contexts are limited, and further studies are warranted to understand the mechanisms through which DPP-4 inhibitors mediate beneficial actions.

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REFERENCES


AUTHOR CONTRIBUTIONS


DISCLOSURES

M. Nangaku received honoraria from Takeda and Astra Zeneca.
RENOPROTECTIVE EFFECT OF DPP-4 INHIBITOR


