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Dipeptidyl peptidase-4 inhibitor ameliorates early renal injury through its anti-inflammatory action in a rat model of type 1 diabetes

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ABSTRACT

Introduction: Dipeptidyl peptidase-4 (DPP-4) inhibitors are incretin-based drugs in patients with type 2 diabetes. In our previous study, we showed that glucagon-like peptide-1 (GLP-1) receptor agonist has reno-protective effects through anti-inflammatory action. The mechanism of action of DPP-4 inhibitor is different from that of GLP-1 receptor agonists. It is not obvious whether DPP-4 inhibitor prevents the exacerbation of diabetic nephropathy through anti-inflammatory effects besides lowering blood glucose or not. The purpose of this study is to clarify the reno-protective effects of DPP-4 inhibitor through anti-inflammatory actions in the early diabetic nephropathy.

Materials and methods: Five-week-old male Sprague–Dawley (SD) rats were divided into three groups; non-diabetes, diabetes and diabetes treated with DPP-4 inhibitor (PKF275-055; 3 mg/kg/day). PKF275-055 was administered orally for 8 weeks.

Results: PKF275-055 increased the serum active GLP-1 concentration and the production of urinary cyclic AMP. PKF275-055 decreased urinary albumin excretion and ameliorated histological change of diabetic nephropathy. Macrophage infiltration was inhibited, and inflammatory molecules were down-regulated by PKF275-055 in the glomeruli. In addition, nuclear factor-κB (NF-κB) activity was suppressed in the kidney.

Conclusions: These results indicate that DPP-4 inhibitor, PKF275-055, have reno-protective effects through anti-inflammatory action in the early stage of diabetic nephropathy. The endogenous biological active GLP-1 might be beneficial on diabetic nephropathy besides lowering blood glucose.

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1. Introduction

Diabetic nephropathy has become the most common cause of end-stage renal disease worldwide [1]. Many mechanisms have been proposed to explain the pathogenesis of diabetic nephropathy. Recently, accumulated data have emphasized that an inflammation plays a crucial role in the pathogenesis of diabetic nephropathy [2,3]. Actually, we have shown that inflammatory molecules and mediators are important in the early stage of diabetic nephropathy by using intercellular adhesion molecule-1 (ICAM-1) -deficient mice and macrophage scavenger receptor-A-deficient mice, and by an administration of methotrexate [4–6].

Currently, incretin-based drugs are being used to achieve better glycemic control in patients with type 2 diabetes. GLP-1 receptor agonists that enhance resistance to the degradation by DPP-4 enzyme, strongly and steadily stimulate GLP-1 receptor in the pharmacological level. On the other hand, DPP-4 inhibitors that block the activity of DPP-4, reinforce the endogenous biological actions of incretin.

The incretin receptors have been found in multiple organs including kidney [7]. Previous reports and our study [8] revealed that GLP-1 receptor was expressed in glomeruli and renal tubule. To our knowledge, gastric inhibitory polypeptide (GIP) receptor was not expressed in the kidney [9]. We studied the effects on diabetic nephropathy animal model by using GLP-1 receptor agonist called exendin-4. Exendin-4 reduced the urinary albumin

Abbreviations: DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; SD, Sprague–Dawley; NF-κB, nuclear factor-κB; GIP, gastric inhibitory polypeptide; ICAM-1, intercellular adhesion molecule-1; PAM, periodic acid-methenamine silver; cAMP, cyclic AMP; CRP, c-reactive protein; IL, interleukin; CD, cluster of differentiation; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α.

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excretion, and attenuated the histological parameters of glomerular injuries characterized by mesangial extracellular matrix expansion and glomerular hypertrophy. It also attenuated inflammatory molecules and mediators, such as ICAM-1, macrophage infiltration, cytokines and NF-κB activity, in the kidney. Furthermore, the effects were shown, observed directly through the GLP-1 receptor in culture cells [8]. The other groups also presented that GLP-1 receptor agonist was beneficial on diabetic nephropathy [10–12].

Recently, the other investigator reported that DPP-4 inhibitor ameliorated diabetic nephropathy by inhibition of apoptosis and sclerosis [13]. Therefore, the endogenous biological effects of GLP-1 would ameliorate renal injuries. However, it is not obvious whether DPP-4 inhibitor prevents the exacerbation of diabetic nephropathy through anti-inflammatory effects in the early stage model of diabetic nephropathy. The purpose of this study is to clarify the reno-protective effects of DPP-4 inhibitor through anti-inflammatory actions in the early diabetic nephropathy.

2. Methods

2.1. Animals

Male SD rats (Charles River, Yokohama, Japan) were purchased from Charles River (Yokohama, Japan). SD rats aged 4 weeks were divided into the following groups: negative control group, non-diabetes (NDM); positive control group, diabetes (DM); and test group, diabetes treated with DPP-4 inhibitor (DM + D) ($n = 7$ per group). At the age of 5 weeks, rats chosen for the DM and DM + D groups were injected intravenously with streptozotocin (Sigma–Aldrich, St. Louis, MO, USA) at 65 mg/kg body weight in citrate buffer (pH 4.5). Only rats with blood glucose concentrations >300 mg/dl at 7 days after streptozotocin injection were used in the diabetes groups. The NDM group received injections of citrate buffer alone. The DM + D group was given DPP-4 inhibitor (PKF275-055 [14]; Novartis, Basel, Switzerland) orally at 3 mg/kg body weight daily for 8 weeks, starting at 1 week after streptozotocin injection. All rats had free access to standard chow and tap water. All procedures were approved by the Committee for Ethics and Animal Experimentation of Nihon Bioresearch Inc. All rats were killed at 9th week after the induction of diabetes, and the kidneys were harvested.

2.2. Metabolic variables

Systolic blood pressure was measured by tail-cuff plethysmography (Softron, Tokyo, Japan). Food intake was calculated as the average over 3 days. Urine samples were collected over a 24 h period in individual metabolism cages. Urinary albumin excretion was measured by nephelometry using anti-rat albumin antibody (ICN Pharmaceuticals, Aurora, OH, USA). Creatinine clearance (ml min^{-1} kg^{-1}) was calculated as described previously [15]. Serum active GLP-1 levels were measured by using ELISA kit (AKMGP-011, Shibayagi, Gunma, Japan) at the pre-prandial (after about 12 h of fasting) and post-prandial (2 h) time. 24 h urinary cyclic AMP (cAMP) excretion was measured by using cAMP Complete Enzyme Immunometric Assay kit (Enzo Life Sciences, Ann Arbor, USA) according to the manufacturer's instructions. Serum c-reactive protein (CRP) levels were measured by rat CRP ELISA kit (Life Diagnostics, PA, USA) according to the manufacturer's instructions. Serum interleukin (IL)-6 levels were measured by rat IL-6 ELISA kit (Uscn Life Science, China) according to the manufacturer's instructions.

2.3. Light microscopy

Periodic acid-methenamine silver (PAM)-stained slice were analyzed as described previously [8]. To evaluate the glomerular size and mesangial matrix area, we examined randomly selected ten glomeruli per animal ($n = 7$ per group). Quantitative analysis for all staining was performed in a blinded manner.

2.4. Immunoperoxidase staining

Immunoperoxidase staining was performed as described previously [16]. Primary antibody was monoclonal antibody against rat monocytes/macrophages (ED1, 1:50; Serotec, Oxford, UK), which was applied for 12 h at 4 °C. Secondary antibody was biotin-labelled goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA, USA) for 60 min at room temperature. Intraglomerular ED1-positive cells were counted in ten glomeruli per animal ($n = 7$ per group). Quantitative analysis for all staining was performed in a blinded manner.

2.5. RNA extraction, quantitative real-time PCR

Total RNA was extracted from glomeruli isolated by the mechanical sieving technique as previously reported [17], and by using a kit (RNeasy plus Mini; Qiagen, Valencia, CA, USA). Real-time PCR was performed as described previously [8]. The amount of PCR products was normalized with β-actin. The specific oligonucleotide primer sequences are shown in Supplementary Table S1.

2.6. Cytokines and chemokines in the kidney

High-throughput multiplex immunoassays in the renal cortex were performed with the Procarta cytokine assay kit (Panomics Inc., CA, USA) according to the manufacturer's instructions, and analyzed by using Bio-plex (Bio-Rad, Tokyo, Japan).

2.7. Nuclear factor-κB activity

Nuclear proteins were extracted from the kidney tissues with a nuclear extract kit (Active motif, Carlsbad, CA) according to the manufacturer's instructions. NF-κB p65-dependent DNA-binding activity was determined by TransAM NF-κB p65 (Active motif) according to the manufacturer's instructions.

2.8. Statistical analysis

All values are expressed as the means ± SEM. Differences between groups were examined for statistical significance by using one-way ANOVA followed by Scheffe's test. For comparisons between two groups, an un-paired t test was used to assess statistical significance. A P value < 0.05 was considered statistically significant.

3. Results

3.1. Metabolic characteristics of experimental animal models

At 8th week after induction of diabetes, HbA1c, food intake, creatinine clearance and kidney weight were elevated to the same level in both the diabetic groups compared with the NDM group (Table 1). Body weight was decreased to the same level in both the diabetic groups. However, there was no significant difference between the DM and DM + D groups. Systolic blood pressure remained at the same level in the three groups. It is noteworthy that PKF275-055 treatment significantly reduced urinary albumin excretion compared with the DM group.

In this experimental animal model, serum active GLP-1concentration was significantly increased in the DM + D group compared with the NDM group at both the pre-prandial and post-prandial

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