Exenatide Reduces Urinary Transforming Growth Factor- β_1 and Type IV Collagen Excretion in Patients with Type 2 Diabetes and Microalbuminuria

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Key Words

Exenatide • Diabetic nephropathy • Urinary biomarkers • Transforming growth factor- β_1 • Type IV collagen

Abstract

Aims: It was reported that exenatide ameliorated renal injury in diabetic rats. The present study was carried out to evaluate the effect of exenatide on 24-hour urinary albumin, urinary transforming growth factor- β_1 (TGF- β_1) and type IV collagen excretion in patients with type 2 diabetes and microalbuminuria. **Methods:** 31 type 2 diabetic patients with microalbuminuria were randomly allocated to receive exenatide (group Exe, n = 13) or glimepiride treatment (group Glm, n = 18) for 16 weeks. Body mass index (BMI), fasting plasma glucose, 2-hour postprandial plasma glucose, glycosylated hemoglobin A_{1c}, systolic blood pressure, diastolic blood pressure, 24-hour urinary albumin, urinary TGF- β_1 and type IV collagen concentration were analyzed between the two treatment groups. 20 age- and BMI-matched healthy subjects were chosen as the normal control group (group NC, n = 20). **Results:** After 16 weeks of treatment, 24-hour urinary albumin, urinary TGF- β_1 and type IV collagen in group Exe were significantly lower than those of group Glm (p < 0.01), while glycemic control had no statistical difference between the two groups. **Conclusions:** Our results indicate that ex-

enatide reduces urinary TGF- β_1 and type IV collagen excretion in patients with type 2 diabetes and microalbuminuria, which may be partly contributory to its directly renoprotective role.

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Introduction

Diabetic nephropathy (DN) is now the most common cause of end-stage renal disease worldwide [1], while the number of patients with diabetes is increasing dramatically in China and other countries around the world [2, 3]. Patients with DN are at a high risk of mortality, mostly from cardiovascular disease events [4]. Therefore, renal-targeted intervention measures designed to decrease proteinuria and delay progression of DN can reduce cardiovascular disease risk and mortality [5, 6].

Studies showed that the scope of matrix accumulation in glomeruli and interstitium correlated strongly with the extent of renal insufficiency and proteinuria [7]. Mediators of increased synthesis or decreased degradation of matrix molecules in diabetic kidney disease are therefore

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of considerable interest. Transforming growth factor- β_1 (TGF- β_1) is a prominent one of these mediators because it stimulates renal cell hypertrophy and promotes extracellular matrix accumulation including type I and type IV collagen, the two hallmarks of DN [8].

Exenatide, a synthetic incretin-mimetic peptide, is currently considered an attractive agent for the treatment of type 2 diabetes mellitus (T2DM). It displays biological properties similar to human glucagon-like peptide-1 (GLP-1), a regulator of insulin secretion and glucose metabolism. It shares approximately 53% homology with the mammalian incretin GLP-1 and binds to and activates GLP-1 receptor (GLP-1R) cloned from islet cells, gut, hypothalamus, and kidney [9–12]. Exenatide increases glucose-dependent insulin secretion by the islet β -cell, inhibits inappropriately elevated glucagon secretion, delays gastric emptying, suppresses production of glucose by the liver, and regulates central feeding, although the mechanisms of action are still under study. Recent studies demonstrated that GLP-1R agonists can ameliorate DN together with the improvement of metabolic anomalies in db/db mice, and can prevent disease progression in early DN through direct effects on the GLP-1R of kidney tissue in type 1 diabetic rats [11, 13]. To explore renoprotective mechanisms of exenatide, we observed its effects on urinary TGF- β_1 and type IV collagen in patients with T2DM and microalbuminuria.

Patients and Methods

Subjects

Written approval for the study was obtained from the Ethics Committee of Huaian First Hospital Affiliated to Nanjing Medical University. Informed consent was obtained from each participant.

Forty-two type 2 DN and 20 healthy volunteers were recruited. Diabetic patients were recruited from the Department of Endocrinology, Huaian First Hospital Affiliated to Nanjing Medical University, who were all hospitalized patients. Healthy subjects were recruited from the Checkup Center, Huaian First Hospital Affiliated to Nanjing Medical University and were not receiving any medications and had no dyslipidemia, fatty liver by B ultrasound examination. Inclusion criteria for diabetic patients were as follows: (1) all patients with T2DM met the criteria of the 1999 WHO diagnosis and classification, (2) had a glycosylated hemoglobin (HbA_{1c}) value of 7.0–10.0%, and (3) had a 24-hour urinary albumin level of 30–300 mg/24 h after determination of two samples. Exclusion criteria comprised patients with a particular history of illness, physical examination or laboratory evidence who (1) had an insulin injection for more than a week in the last 3 months, (2) had statins, angiotensin II receptor blocker, angiotensin-converting enzyme inhibitors in the previous 2 weeks, (3) had heart disease, liver disease, cerebrovas-

cular disease, or rheumatic disease, (4) had primary nephropathy or other secondary kidney diseases, and (5) had acute diabetic complications.

Methods

Forty-two patients were randomly assigned to exenatide (group Exe, $n = 19$) and glimepiride treatment (group Glm, $n = 23$), all combined with metformin 1.0–1.5 g/day, diet and appropriate exercise therapy. The patients in group Exe received exenatide and metformin, exenatide was initiated at 5 μ g administered twice daily within the 60 min before breakfast and dinner in the first 4 weeks, the dose was increased to 10 μ g twice daily in the subsequent 12 weeks. The patients in group Glm received glimepiride (1–4 mg once daily) and metformin. The dose of glimepiride and metformin was adjusted in accordance with the levels of blood glucose. Exenatide was provided by Eli Lilly & Co. All diabetic subjects were asked to keep their fasting plasma glucose (FPG) between 4.4 and 8.0 mmol/l and maintain the 2-hour postprandial plasma glucose (2h-PG) <11.1 mmol/l. Otherwise, they were excluded. After 16 weeks of observation, 6 patients withdrew from group Exe because of uncontrolled blood glucose and drug side effects including nausea, vomiting, diarrhea, or dizziness, while 5 patients withdrew from group Glm because of loss to follow-up or uncontrolled blood glucose. Therefore, a total of 31 patients with T2DM and microalbuminuria including 13 in group Exe and 18 in group Glm were included in the 16-week study. 20 age- and body mass index (BMI)-matched healthy subjects were chosen as the normal control group (group NC). Baseline characteristics of the patients are given in table 1.

Venous blood was drawn to measure the levels of FPG, 2h-PG and HbA_{1c} after an overnight fast. Weight was measured at the start and the end of the study. The levels of blood glucose, systolic blood pressure (SBP), diastolic blood pressure (DBP) were determined every 2 weeks. 24-Hour urine was collected twice, the average of the two results was considered as 24-hour urinary albumin level. Urine (4 ml) was preserved at -70°C to test albumin, TGF- β_1 , and type IV collagen concentration. All the above parameters were repeated after 16 weeks of treatment. Urinary TGF- β_1 was determined using sandwich enzyme immunoassay (Quantikine kit for human TGF- β_1 immunoassay; R&D Systems, Minneapolis, Minn., USA); the inter- and intra-assay coefficients of variation were less than 10%. Urinary type IV collagen was determined with enzymatic immunoassay kits (Daiichi Fine Chemical Co. Ltd, Japan) according to the manufacturer's instruction [14, 15]; the inter- and intra-assay coefficients of variation were below 10%. The values of all other parameters were obtained by routine standard measurements. To eliminate the possible impact of urine volume, urinary TGF- β_1 and type IV collagen results were corrected for urine creatinine.

Statistics

The Kolmogorov-Smirnov normality test was used to analyze the distribution of all quantitative data. Normally distributed data were expressed as means $\pm$ SD and analyzed by SPSS software, version 13.0 (SPSS, Inc., Chicago, Ill., USA). Non-parametric statistics was used if data was not normally distributed. The paired t test was used to evaluate the differences between pre- and post-treatment, whereas the differences between the groups were assessed by independent-sample t test. A p value <0.05 was considered statistically significant.

Table 1. Baseline characteristics of subjects

Variable	Group Exe	Group Glm	Group NC
Cases (m/f)	13 (10/3)	18 (13/5)	20 (18/6)
Age, years	50.2 ± 14.3	52.0 ± 15.0	49.7 ± 14.3
Diabetes diagnosed, years	4.2 ± 3.2	4.1 ± 2.9	–
BMI	24.9 ± 2.0	24.8 ± 2.0	23.8 ± 2.1
FPG, mmol/l	9.1 ± 1.6*	9.7 ± 2.3*	4.9 ± 0.7
2h-PG, mmol/l	14.8 ± 3.7*	14.5 ± 2.4*	6.5 ± 0.9
HbA _{1c} , %	8.7 ± 1.0	9.0 ± 0.9	–
SBP, mm Hg	136 ± 13*	139 ± 15*	118 ± 15
DBP, mm Hg	82 ± 9*	82 ± 9*	73 ± 8
24-UAE, mg/day	107 ± 71	111 ± 74	–

Data are expressed as mean ± SD. BMI = Body mass index; FPG = fasting plasma glucose; 2h-PG = 2-hour postprandial plasma glucose; HbA_{1c} = glycosylated hemoglobin; SBP = systolic blood pressure; DBP = diastolic blood pressure; 24-UAE = 24-hour urinary albumin excretion.

* $p < 0.01$, diabetes patients vs. healthy subjects.

Table 2. Changes in metabolic variables before and after treatment

	Group Exe		Group Glm	
	before treatment	after treatment	before treatment	after treatment
BMI	24.9 ± 2.0	23.3 ± 1.2*	24.8 ± 2.0	24.7 ± 1.6
FPG, mmol/l	9.1 ± 1.6	7.2 ± 0.8*	9.7 ± 2.3	7.0 ± 0.7*
2h-PG, mmol/l	14.8 ± 3.7	9.3 ± 1.0*	14.5 ± 2.4	9.3 ± 1.2*
HbA _{1c} , %	8.7 ± 1.0	7.4 ± 0.6*	9.0 ± 0.9	7.4 ± 0.6*
SBP, mm Hg	136 ± 13	132 ± 9*	139 ± 15	137 ± 10
DBP, mm Hg	82 ± 9	81 ± 9	82 ± 9	81 ± 6

Data are expressed as mean ± SD.

* $p < 0.01$ vs. before treatment.

Results

Comparison of Baseline Characteristics among the Three Groups

All quantitative data including those for the urinary measurements were normally distributed, so they were expressed as means ± SD. As shown in table 1, there were no significant differences in age, sex, BMI, FPG, 2h-PG, HbA_{1c}, SBP, DBP, or 24-hour urinary albumin between the two treatment groups, while the differences in FPG and 2h-PG were significant between diabetic patients and healthy subjects.

Fig. 1. Percentage change of BMI, SBP, FPG, 2h-PG, and HbA_{1c} before and after treatment in group Exe and group Glm.

Changes of BMI, FBG, 2h-PG, HbA_{1c}, SBP and DBP in the Two Groups before and after Treatment

As shown in table 2 and figure 1, the levels of BMI in group Exe decreased by 5.95% from 24.9 to 23.3 kg/m² after therapy, while the reduction in group Glm was not significant, from 24.8 to 24.7 kg/m², or 0.25% decrease. In addition, a significant decrease of FPG, 2h-PG, and HbA_{1c} was observed in the two groups after treatment, but the decreased percentage between the two groups was not significant. We also observed a decrease of SBP in group Exe after treatment, but not in group Glm. However, the percentage change of SBP between the two groups was not statistically different (fig. 1; 2.76% decrease in group Exe vs. 1.10% decrease in group Glm). The levels of DBP between the two groups had no significant change after treatment.

Exenatide Reduced Urinary Excretion of Albumin, TGF-β₁ and Type IV Collagen

Table 3 and figure 2 show the changes and percentage change of urinary albumin, TGF-β₁ and type IV collagen in the two treatment groups. The levels of 24 h urinary albumin in group Exe dropped significantly by 37.97% from 107 to 65 mg/l after 16 weeks of treatment ($p < 0.01$), while the reduction in group Glm was not significant, from 111 to 106 mg/l, or 5.76% decrease. The levels of urinary TGF-β₁ were significantly lower by 37.3% from 178 to 109 ng/g Cr after 16 weeks of exenatide treatment ($p < 0.01$), while in group Glm there was no improvement,

Fig. 2. Percentage change of 24-hour urinary albumin excretion (24-UAE), urinary TGF- β_1 (uTGF- β_1) and type IV collagen (uIV-Col) excretion at baseline and 16 weeks after treatment in group Exe and group Glm.

from 173 to 175 $\mu\text{g/g Cr}$, or 1.45% increase. Similarly, excretion of urinary type IV collagen in group Exe decreased significantly after treatment ($p < 0.01$), while it was slightly without statistical significant in group Glm. The decreased magnitude of urinary type IV collagen in group Exe was significantly different from group Glm (fig. 2; 25.3% decrease in group Exe vs. 1.6% decrease in group Glm; $p < 0.005$).

Discussion

In the present study, we demonstrated for the first time that exenatide treatment can lower the excretion of urinary TGF- β_1 and type IV collagen in patients with T2DM and microalbuminuria, and exenatide treatment reduced BMI and SBP, which might contribute to its renoprotection beyond the hypoglycemic effect.

Exenatide is a synthetic version of exendin-4, a hormone found in the saliva of the Gila monster that was first isolated by Dr. John Eng in 1992 [16]. It displays biological properties similar to human GLP-1 which is produced by L endocrine cells of the intestine following ingestion of food [17]. GLP-1 stimulates the synthesis and secretion of insulin from islet β -cells via G-protein-coupled GLP-1R, and it promotes islet β -cell proliferation and inhibits apoptosis [18]. Previous studies showed that GLP-1R is localized not only in the pancreas, gut, brain, heart, lung,

Table 3. Changes in urinary TGF- β_1 and type IV collagen profile

	Group Exe		Group Glm	
	before treatment	after treatment	before treatment	after treatment
24-UAE, mg/day	107 $\pm$ 71	65 $\pm$ 47*	111 $\pm$ 74	106 $\pm$ 75
uTGF- β_1 , ng/g Cr	178 $\pm$ 44	109 $\pm$ 24*	173 $\pm$ 33	175 $\pm$ 34
uIV-Col, $\mu\text{g/g Cr}$	7.7 $\pm$ 2.5	5.6 $\pm$ 1.6*	7.9 $\pm$ 2.2	7.5 $\pm$ 1.8

Data are expressed as mean $\pm$ SD. uTGF- β_1 = Urinary TGF- β_1 ; uIV-Col = urinary type IV collagen.

* $p < 0.01$ vs. before treatment.

liver, muscle cells, and adipocytes, but also in the kidney, and the discovery of GLP-1R outside of the islet β -cell presents direct evidence that GLP-1 has many extrapancreatic functions [11, 19]. With respect to the functions of GLP-1R on the kidney, it has been reported that exendin-4 prevented disease progression of early DN through GLP-1R in kidney tissue and suppressed advanced glycation end products (AGE)-induced monocyte chemoattractant protein-1 (MCP-1) secretion via activating GLP-1R in mesangial cells. Clinical studies demonstrated that exenatide improves glycemic control, reduces body weight, and improves β -cell function and insulin sensitivity in patients with T2DM [20]. However, the effects of exenatide on patients with DN are still unknown. Our current study in a Chinese population was conducted to examine the effects and the basic mechanisms of exenatide in patients with T2DM and microalbuminuria.

Previous studies suggested that obesity is associated with a new onset of chronic kidney disease and progression to kidney failure in animals and humans [21]. The pathophysiology of obesity-associated renal damage includes the early onset of glomerulomegaly, hemodynamic obstacles of hyperfiltering kidney, and increased albuminuria. BMI levels in the Asian population and diabetic patients are generally lower than those in Western countries. The WHO has made lower BMI cut-off points for overweight ($>23.0 \text{ kg/m}^2$) and obesity ($>25.0 \text{ kg/m}^2$) in Asians. Similarly, our results showed BMI levels of patients were $<25 \text{ kg/m}^2$. In this study, we found that exenatide treatment markedly reduced BMI and 24-hour urinary albumin excretion. The results showed the possibility that renoprotective effects of exenatide came from decreased weight.

It is widely known that increased systemic and intraglomerular pressure are implicated in the pathogenesis of

DN. High SBP as well as the intraglomerular hypertension promotes albuminuria, which induces secretion of local proinflammatory and profibrotic cytokines resulting in renal damage. Recent studies demonstrated that exenatide treatment ameliorated hypertension by regulation of sodium excretion and inhibition of angiotensin II-induced high-salt sensitivity in tubular cells [22, 23]. In our study, we also showed that exenatide, not glimepiride, significantly decreases SBP level, which indicates a possible benefit of exenatide on DN.

Hyperglycemia and hyperglycemia-induced metabolic and hemodynamic factors are recognized to be major mediators of kidney injury and could enhance TGF- β levels. TGF- β is a profibrotic and proinflammatory growth factor involved in the expansion of mesangial matrix and glomerula hypertrophy. Urinary TGF- β and type IV collagen levels have been proven to correlate with the severity of microalbuminuria [24]. Park et al. [13] showed that exendin-4 treatment significantly improved glomerular hypertrophy, mesangial matrix expansion, TGF- β_1 expression, and type IV collagen accumulation. Kodera et al. [11] reported that exendin-4 ameliorates renal injury through decreasing the expression of TGF- β_1 and type IV collagen in the kidney and inhibiting production of intercellular adhesion molecule-1 without lowering blood glucose in type 1 diabetic rats. Ishibashi et al. [25] confirmed that GLP-1 decreased ROS genera-

tion and reduced MCP-1 gene and protein expression on AGE-exposed mesangial cells. In this study, our results showed that exenatide treatment had a similar effect on FPG, 2h-PG, and HbA_{1c} with glimepiride treatment, but a remarkable decrease in urinary TGF- β_1 and type IV collagen levels. It indicated that exenatide decreased urinary excretion of TGF- β_1 and type IV collagen independent of its hypoglycemic effect.

In conclusion, our current study shows that exenatide may significantly reduce urinary albumin, TGF- β_1 and type IV collagen excretion in patients with T2DM and microalbuminuria, and the effects were not entirely dependent on glycemic control. The results suggested that exenatide could provide a renoprotective role in patients with T2DM and microalbuminuria beyond the hypoglycemic effect. However, the exact mechanisms need to be further explored.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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