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EXPERIMENTAL STUDY

Glucagon-like peptide-1 improves mesangial proliferative glomerulonephritis in rats

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ABSTRACT

OBJECTIVE: To investigate the role of GLP-1 in rat with mesangial proliferative glomerulonephritis.

METHODS: A total of 27 normal male Sprague-Dawley rats were randomly divided into 3 groups: NC group (normal control group); MC group (nephritis model rats) and GLP-1 group (nephritis rats treated with GLP-1) with 9 rats in each group. 24-hour urinary protein excretion, serum cystatin C and serum creatinine were tested at the end of 12 weeks. The proliferation of mesangial cells was measured by H&E and TUNEL staining of paraffin sections. The expressions of IKK-β, NF-κB, IL-6 and TNF-α were detected by immunohistochemisty and RT-PCR.

RESULTS: Compared with NC group, 24-hour urinary protein excretion, serum cystatin C and creatinine in MC groups significantly increased ($p < 0.05$, respectively), the 24-hour urinary protein excretion, serum cystatin C and creatinine in GLP-1 group were significantly decreased compared with MC group ($p < 0.05$, respectively). The relative protein and gene expressions of IKK-β, NF-κB, IL-6 and TNF-α of MC group were significantly up-regulated compared with NC group ($p < 0.05$, respectively). However, compared with MC group, the IKK-β, NF-κB, IL-6 and TNF-α gene and proteins expression were significantly suppressed in GLP-1 group ($p < 0.05$, respectively).

CONCLUSION: The mesangial glomerulonephritis is correlated with GLP-1 and GLP-1 up-regulation had effects to suppress mesangial glomerulonephritis by IKK-β pathway *(Fig. 4, Ref. 22)*. Text in PDF www.elis.sk.

KEY WORDS: glomerulonephritis, membranoproliferative, GLP-1, IKK-β pathway.

Introduction

Glucagon like peptide-1 (GLP-1) is a polypeptide containing 30 amino terminal ileum, jejunum, colon epithelial cells secrete L, modified after the GLP.1 receptor agonist half-life is prolonged, is a kind of new antidiabetic drugs. The effect of GLP-1 receptor agonist on hypoglycemic effects showed that GLP-1 has anti-inflammatory effects in different tissues including human umbilical vein endothelial cells, glomerular endothelial cells, monocytes and macrophages (1–3). IKK-β/NF-κB pathway is a chronic inflammation signal transduction pathway important in the process of development, and a variety of models of inflammation are closely related (4–6). However, it has been not clear that the effects of GLP-1 in nephritis rat model and GLP-1 correlated with IKK-β/NF-κB pathway in nephritis rat model. In our present study, we wanted to study and explain the effects and mechanism of GLP-1 in nephritis rat model in vivo study.

Materials and methods

Laboratory animals

48 healthy and clean grade SD male rats , with a weight 200 ± 10 g were purchased from Nanjing Medical University. Before the experiment the rats were fed by normal diet for 1 week and were freely eating and drinking.

Reagent

Bovine serum albumin (BSA), lipopolysaccharide (LPS), carbon tetrachloride (CCl4), castor oil and GLP-1 were purchased from Sigma Corporation of the United States, Rabbit anti rat IKK-β, NF-κB, IL-6 and TNF-α, immunohistochemical SP assay kit was purchased from Biological Engineering Co., Ltd. In Wuhan, and the reverse transcription polymerase chain reaction (RT-PCR) kit was purchased from Beijing Ding Sheng biotechnology limited liability Company.

Experiment methods

After the 27 rats were suitable fed for 1 week; the body weights were measured and the rats were sorted by body weight. The rats were randomly divided into 3 groups: NC group (normal control group); MC group (Model control group) and GLP-1 group (GLP-1 injection group). NC and MC groups were fed by gavage with same dose of distilled water and continued for 12 weeks. After feeding for 12 weeks, the 24-h urine samples of the different groups were collected by metabolic cage to measure 24-h urine protein. After that, fasting overnight, 10 % chloral hydrate intraperitoneal anesthesia after thoraco-abdominal incision, 5 ml blood was directly taken from the inferior vena cava, than centrifuged at 3000 g /min for 10 min, serum creatinine was measured by automatic biochemical analyzer (Scr), serum cystatin C (Cys-C) level was assessed, the left renal cortex was fixed with 10 % formalin, then followed

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a regular ethanol dehydration, xylene, the sample was embedded in paraffin, 2 μm slices were used for HE, then TUNEL staining and immunohistochemistry and resection of the right kidney were performed and the tissue was frozen at –80 °C in a refrigerator for RT-PCR detection.

Item testing

Immunohistochemistry

Paraffin sections were routinely dewaxed to water, microwave heat repair antigen, 3 % H_2O_2 was added to block endogenous peroxidase, goat serum closed non-specific antigen, Rabbit anti mouse IKK-β polyclonal antibody (1: 5), Rabbit anti mouse NF- κB antibody (1:75), Rabbit anti mouse TNF-polyclonal antibody (1.100), Rabbit anti mouse IL-6 polyclonal antibody (1.100) was added, cultured at 4 °C overnight, horseradish peroxidase labeled with anti- rabbit IgG was added, DAB color, phosphate buffer (PBS) instead of the first antibody as negative control was used, brown granular material as positive markers was used, under high magnification according to the degree of cell membrane and cytoplasmic staining the numbers of positive cells and total cells were counted.

RT-PCR

Primers are synthesized in Beijing Ding Sheng biotechnology limited liability Company. Primer sequence: IKK-β: F: 5'-CATTGTTGTTAGCGAGGAC-3'; R: 5'-CTTTGCCGAGGTTGC-3'; NF-κB: F:5'- AATTTGGCTTCCTTTCTTGGCT-3'; R: 5'-CTGCGATACCTTAATGACAGCG-3'; IL-6: F:5'- CCACTGCCTTCCCTACTTC-3' ; R: 5'-CTGGCTTTGTCTTTCTTGTTA-3'; TNF-α: F: 5'-ACTGGCGTGTTCATCCG-3'; R: 5'-CCACTACTTCAGCGTCTCGT-3'; β-actin: F: 5-GAAATCGTGCGTGACATTA-3', R: 5'-TAGGAGCCAGGGCAGTAA-3'. According to following: 94°C for 5min, 94 °C for 30 s, 56 °C for 30s, 75 °C for 30 s, after 35 cycles, 72 °C for 5min.

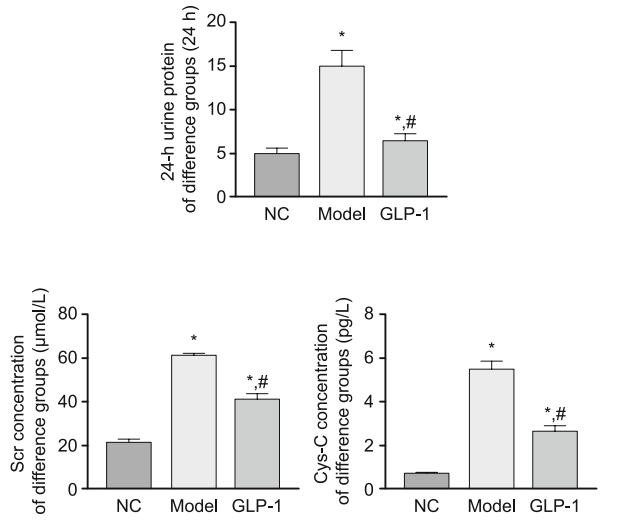

Fig. 1. The 24-h Urine protein and blood biochemistry of the different groups. * p<0.05, compared with NC group, # p<0.05, compared with Model group.

Renal pathological glomerular score

(1) The proliferation of mesangial cells: mesangial area has 3 mesangial cells counted as 1 point, 4 mesangial cells counted as 2 points, more than 5 cells counted as 3 points.

(2) The widening of mesangial matrix: matrix increased width does not exceed the capillary diameter, segmental distribution showed, capillary cavity remains open is 1 point, matrix widened slightly more than the capillary diameter, diffuse distribution, hyperplasia of oppression and destruction are counted as 2 points on the glomerular capillary loop. Matrix widened diffuse and segmental hyperplasia aggravation, seri-

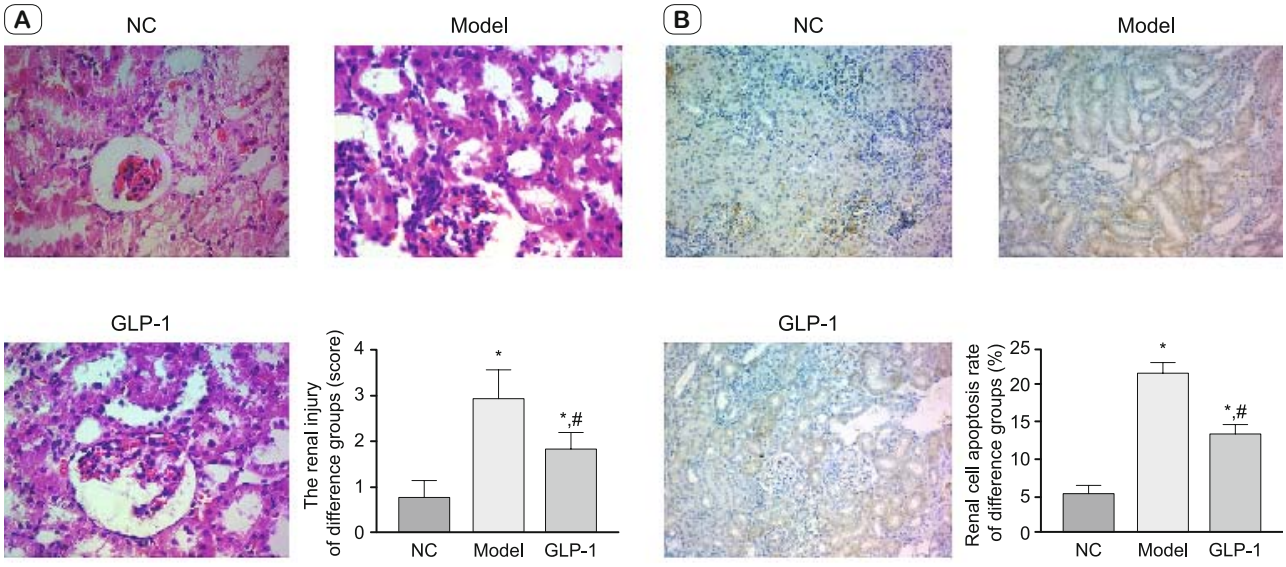

Fig. 2. The renal pathology and renal cell apoptosis of the different groups. A) The renal pathology of the different groups by H&E staining, B) The renal cell apoptosis of the different groups by TUNEL assay. * p<0.05, compared with NC group, # p<0.05, compared with Model group.

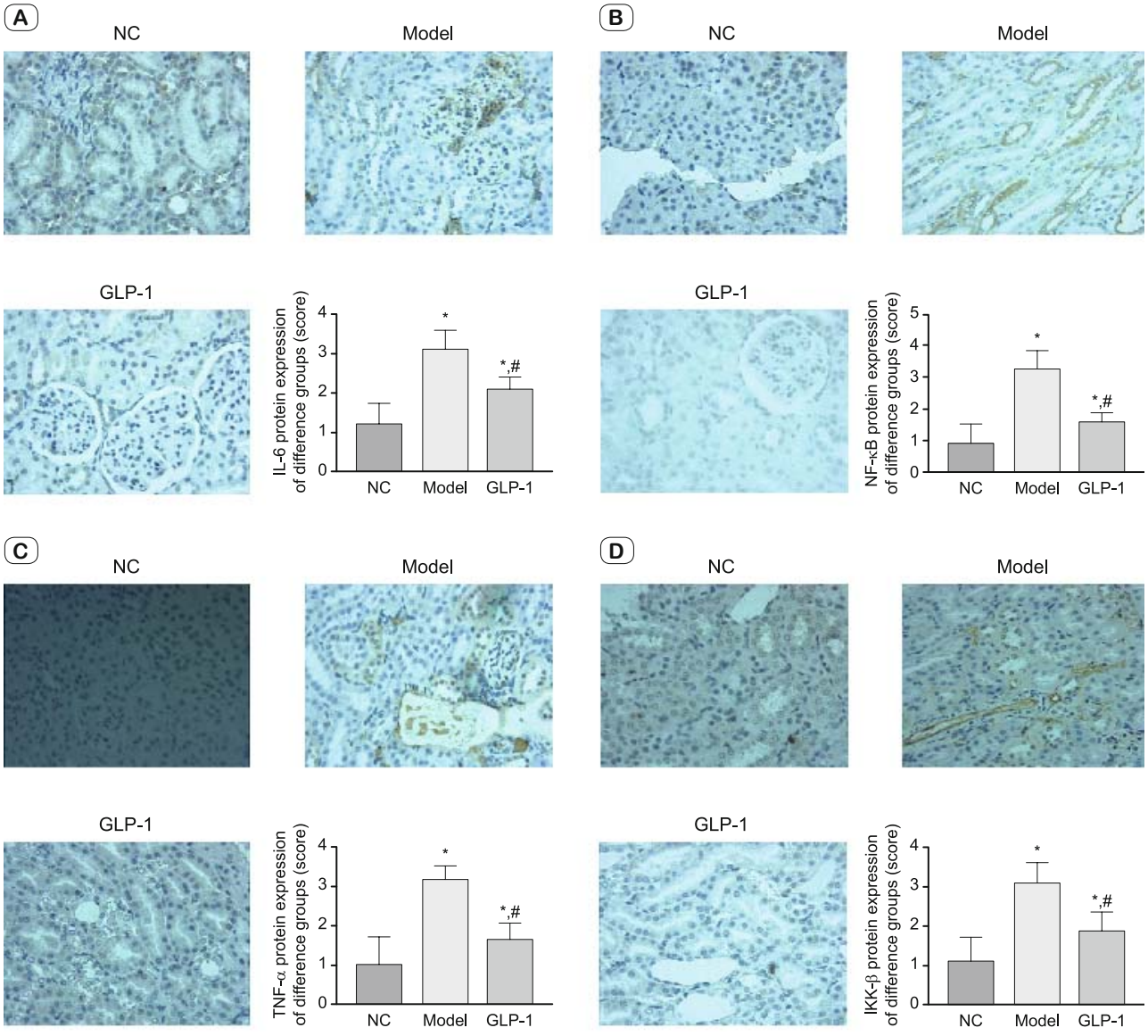

Fig. 3. The relative proteins expressions of the different groups. A) The IL-6 protein expression of the different groups by IHC, B) The NF-κB protein expression of the different groups by IHC, C) The TNF-α protein expression of the different groups by IHC, D) The IKK-β protein expression of the different groups by IHC. * p<0.05, compared with NC group, # p<0.05, compared with Model group.

ous oppression and destruction phenomenon is counted as 3 points on the glomerular capillary loop.

(3) Sclerosis

(4) Stenosis and destruction of the capillary loops.

(5) Basement membrane thickening.

(6) Adhesion of saccule.

(3)~(6) scoring standard: The lesions ranged from 30 % to 1, and the lesion ranged from 30 % to 60 %, with a lesion range of 60 % and over 3, 2. 10 glomeruli were observed at 400 times under each microscope, and the average number was counted as the integral score of glomerular pathology, with a total score of 0~18. Less than 6 points is mild damage, 7 to 11 points is moderate damage, more than 12 points is severe damage.

Statistical analysis

All data were analysed by SPSS 20.0 software. The data are displayed as mean ± standard deviation (SD). The statistical significance of differences was determined by two-way ANOVA, followed by Bonferroni's multiple comparisons test, as appropriate. $p < 0.05$ was considered statistically significant.

Results

The 24-h urine protein of the different groups

Compared with NC group, the 24-h Urine protein of Model and GLP-1 group were significantly increased ($p<0.05$, respectively). However, the 24-h urine protein concentration of GLP-1

group was significantly down-regulated compared with that of Model group ($p<0.05$). The relative data are shown in Figure 1.

The blood biochemistry of the different groups

Compared with NC group, the serum Scr and Cys-C concentration levels of Model and GLP-1 groups were significantly up-regulated ($p<0.05$, respectively). Meanwhile, there were significant differences between Model and GLP-1 groups ($p<0.05$, respectively). The relative data are shown in Figure 1.

Renal pathology of the different groups

The rats of NC group had no obvious changes in the glomeruli and tubules and the arrangement, distribution and morphology were normal. There were mesangial cell proliferation, mesangial matrix thickening, thickening of the cystic wall, dilatation or atrophy of renal tubules, enlargement of renal interstitium, and fibrosis in part of the renal interstitium, and infiltration of inflammatory cells in the Model group observed. Compared with Model group, the Inflammatory symptoms of GLP-1 group were significantly improved ($p<0.05$). The relative data are shown in Figure 2A.

The renal cell apoptosis of the different groups

Based on the TUNEL assay, the renal cell apoptosis rate of Model and GLP-1 groups were significantly increased compared with NC group ($p<0.05$, respectively). However, the renal cell apoptosis rate of GLP-1 group was significantly suppressed compared with that of Model group ($p<0.05$). The relative data are shown in Figure 2B.

The relative proteins expression by IHC

The IKKβ, NF-κB, TNF-α and IL-6 proteins expression of Model and GLP-1 groups were significantly up-regulated compared with those of NC group ($p<0.05$, respectively). Meanwhile, there were significant differences between Model and GLP-1 groups in IKKβ, NF-κB, TNF-α and IL-6 proteins expressions ($p<0.05$, respectively). The relative data are shown in Figure 3.

The relative genes expression of the different groups

The IKKβ, NF-κB, TNF-α and IL-6 genes expression of Model and GLP-1 groups were significantly up-regulated compared with those of NC group ($p<0.05$, respectively). Meanwhile, there were significant differences between Model and GLP-1 groups in IKKβ, NF-κB, TNF-α and IL-6 genes expressions ($p<0.05$, respectively). The relative data are shown in Figure 4.

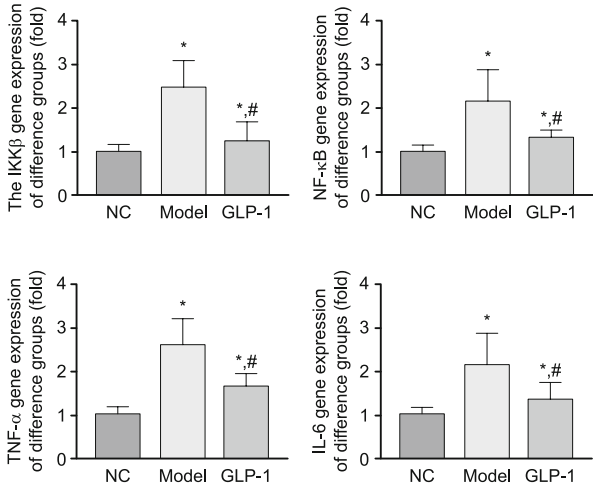

Fig. 4. The relative genes expression of the different groups. * p<0.05, compared with NC group, # p<0.05, compared with Model group.

Discussion

IKKβ/NF-κB signaling pathway has an important role in chronic inflammatory response (4–6). NF-κB widely exists in many kinds of cells, it can regulate the expression of cytokines, chemokines and adhesion factors, and affect the inflammatory reaction in vivo (7). GLP-1 agonist has effects to inhibit NF-κB activity induced by TNF-α. Previous study found that GLP-1 agonist had anti- inflammatory effects via regulation of IKKβ/NF-κB pathway in animal disease models (8–10).

Recent studies have shown that cytokines and inflammatory mediators involved in inflammation in asthma are regulated by NF-κB at the transcriptional level (11, 12). PgaeK et al (13) found that the accumulation of EOS, airway hyper-responsiveness, and airway inflammation were unable to occur after knockout of the NF kappa B gene in mice. Barnes et al (14) found that in patients with asthma and its distribution in bronchial epithelial cells, alveolar macrophages, vascular endothelial cells and submucosal cells the NF-κB activity was significantly increased, the inhibition of NF-κB activity expression can be used in the treatment of asthma. NF-κB was activated by many stimulatory factors, the activation of NF-κB can promote the high expression of some substances, such as cytokines, growth factor, acute protein, chemokines, immune receptors and transcription factors that lead to inflammation, and these products may be used for anti NF-κB activation, so that the local inflammatory response continues to expand (15–17). The present study found that IKK plays a key regulatory role in the NF kappa B signaling pathway, and its status further leads to phosphorylation and degradation of I kappa B protein and activation of NF-κB. NF-κB is found mainly in cells and is bound to its antagonist protein I-κB, which is often inactive. When the cells are stimulated by viruses and bacteria and other sensitive factors, I-κ B two conserved N-terminal serine residues phosphorylation occurs at signal position so that the NF-κB is exposed, activation of NF-κB iss transferred to the nucleus and combined with κB sites of occurrence of specific gene responsible for expression and regulation of inflammatory factors (18–21). IKK consists of 2 catalytic subunits, IKKα, IKKβ, and regulatory subunit IKKγ. Among them, the activation of IKK complex basically depends on the phosphorylation of IKKβ, IKKβ is a necessary subunit of IKK to play a biological function (22).

In our present study, it was shown that GLP-1 had improved the rat mesangial proliferative glomerulonephritis via IKKβ/NF-κB/TNF-α/IL-6 pathway in in vivo study.

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