Effect of saxagliptin on the expression of HIF-1α in the liver of diabetic rats with fatty liver

QIAN ZHAO, QIAN XIAO, XIANGYING MENG, JIANYANG CHEN, WEIWEI YE and YONG ZHOU

Department of Endocrinology, Dahua Hospital of Xuhui District, Shanghai 200237, P.R. China

Abstract. The present study was designed to establish a rat model of type 2 diabetes mellitus (T2DM) complicated with fatty liver and to detect the expression of hypoxia-inducible factor-1α (HIF-1α) in liver tissue during the treatment with saxagliptin. Eighty male Wistar rats were randomly divided into two groups. One group was fed with high fat diet to establish T2DM model (n=40), and the other group was fed normally to serve as the control group (n=40). Successfully established rat T2DM models were randomly divided into two groups: The treatment group that received intraperitoneal injection of saxagliptin solution and the other the model group with normal breeding. Blood glucose, blood lipid, liver function and the expression of HIF-1α in liver tissue were detected. Levels of blood glucose in model treatment group were significantly higher than those in the control group (p<0.05). Levels of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyl transferase (GGT) in model and treatment group were significantly higher than those in control group (p<0.05), but were significantly reduced with the prolonged treatment (p<0.05). Levels of TC, TG, LDL-C, HDL-C, AST, ALT and GGT and expression level of HIF-1α were significantly higher in the model group than in control group before 3 weeks of treatment (p<0.05), but no significant differences were found after 3 weeks of treatment (p>0.05). Expression level of HIF-1α was decreased with the prolonged treatment, and no significant difference in expression level of HIF-1α was found 3 weeks after treatment (p>0.05). In conclusion, HIF-1α is highly expressed in rats with T2DM and fatty liver. Saxagliptin can effectively improve blood glucose, blood lipid and liver function and reduce the protein expression of HIF-1α in diabetic rats with fatty liver.

Introduction

With the changes in people's diet structure, such as the popularization of Western-style diet, incidence of type 2 diabetes mellitus (T2DM) shows an increasing trend (1). Zinman *et al* (2) reported that T2DM affected 3 million patients in 2015. Green *et al* (3) predicted that the number of patients with T2DM will exceed 5 million in 2030. Presently, T2DM is the most common type of chronic disease worldwide (4). T2DM is a chronic metabolic disorder characterized by insufficient insulin secretion or resistance (5). Complications of T2DM mainly include glucose and lipid metabolism disorder, of which fatty liver is the most common complication (6). At present, there is no radical treatment of T2DM in clinical practice, and conventional treatment can only delay the development of T2DM (7). Hypoxia-inducible factor-1α (HIF-1α) is a protein with critical roles in post-transcriptional and post-transcriptional regulation of gene expression. Studies in recent years (8-10) have shown that HIF-1α is closely correlated with the onset and development of T2DM, while its involvement in T2DM-induced fatty liver is unclear. Saxagliptin, as a potent inhibitor of dipeptidyl peptidase-4 (DPP-4), can increase the level of endogenous glucagon-like peptide-1 (GLP-1) to regulate blood glucose. In this study, a rat model of T2DM complicated with fatty liver was established and the expression of HIF-1α in liver tissue during the treatment with saxagliptin was detected. Our study provided new insights for the treatment of T2DM complicated with fatty liver.

Materials and methods

Animals. Eighty male Wistar rats weighted 170-250 gr were provided by the Experimental Animal Center of Hunan Normal University. Feeding conditions: Room temperature 26°C, humidity 75%, five rats in one cage, normal light and free access to water. The study was approved by the Ethics Committee of Dahua Hospital of Xuhui District (Shanghai, China).

Model construction. All rats were randomly divided into two groups. One group was subjected to T2DM modeling (n=40)

Correspondence to: Dr Yong Zhou, Department of Endocrinology, Dahua Hospital of Xuhui District, 901 Laohumin Road, Shanghai 200237, P.R. China
E-mail: yuxinyi0703@126.com

and the other group was control (n=40). Rats in model group were fed with high fat diet (0.5% cholic acid, 1% cholesterol, 15% lard, 20% sucrose, 63.5% basal diet) after 1 week adaptive feeding. After continuous feeding for 4 weeks, intraperitoneal injection of streptozotocin (30 mg/kg, once a day) was performed. After 8 weeks of feeding, fasting blood glucose, insulin sensitivity index and indexes of liver function were detected. Based on the diagnostic criteria for fatty liver proposed in 2015 (11), fasting blood glucose (>16 mmol/l) and the decrease of insulin sensitivity index were used as the indicators of the successfully established models.

Experimental methods. Successfully established rat T2DM models were randomly divided into two groups: One group was treatment group that received intraperitoneal injection of saxagliptin solution (10 mg/kg, once per day), the other was a model group with normal breeding. Six mice in each of the three groups were sacrificed at 12 h after the model construction, 1 and 3 weeks after treatment. Blood (2 ml) was extracted, followed by centrifugation at 3,500 x g for 5 min. Beckman Coulter AU5800 automatic biochemical analyzer (Beckman Coulter, Inc., Brea, CA, USA) was used to detect blood glucose, blood lipids and liver function indexes. Rat liver was removed and fixed in 10% neutral formalin. Expression of HIF-1α protein in liver tissue was detected by western blot analysis.

Western blot analysis. After total protein extraction, 10% PAGE gel electrophoresis was performed, followed by transmembrane under 100 V for 2 h. After blocking for 1 h at room temperature, membranes were incubated with rabbit monoclonal HIF-α antibody (cat. no. ab51608; dilution 1:250) overnight, followed by incubation with secondary goat anti-rabbit (HRP) IgG antibody (cat. no. ab6721; dilution 1:1000) for 1-2 h at room temperature. All the antibodies were all purchased from Abcam (Cambridge, MA, USA). Test was in accordance with BD's test kit instructions (BD Biosciences, Franklin Lakes, NJ, USA).

Observation indicators. Blood glucose, triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C); liver function indexes: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT); HIF-1α protein expression.

Statistical analysis. SPSS 22.0 statistical software (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Enumeration data are expressed as rate and processed using Chi-square test. Measurement data are expressed as mean ± standard deviation, and comparisons among multiple groups and between two groups were performed by analysis of variance followed by Least Significant Difference as its post hoc test and t-test, respectively. P<0.05 indicates a difference with statistical significance.

Results

Results of model construction. Thirty-seven model rats were successfully established, and success rate was 92.5% (37/40).
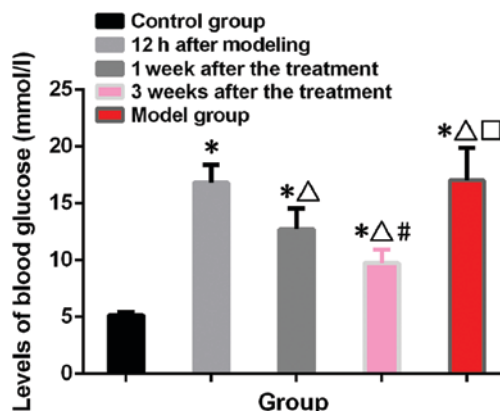

Figure 1. Blood glucose test results of two groups of rats. There was no significant difference between model and treatment group at 12 h after model construction. Levels of blood glucose in model and treatment group were significantly higher than those in control group at 12 h after model construction, and 1 and 3 weeks after treatment. In treatment group, blood glucose gradually decreased with the prolonged treatment. *p<0.05, compared with control group; ᐃp<0.05, compared with 12 h after model construction; #p<0.05, compared with 1 week after treatment; ▢p<0.05, compared with 3 weeks after treatment.

Those rat models were randomly divided into treatment group (n=19) and model group (n=18).

Blood glucose test results. Blood glucose in control group was 5.16±0.24 mmol/l, and blood glucose in model group was 17.04±2.82 mmol/l. Blood glucose levels in treatment group at 12 h after model construction, 1 and 3 weeks after treatment were 16.82±2.56, 12.72±1.84 and 9.73±1.18 mmol/l, respectively. Levels of blood glucose in model and treatment group were significantly higher than those in control group (p<0.05). There was no significant difference between model and treatment group at 12 h after model construction (p>0.05). Blood glucose level 3 weeks after treatment were lower than those 1 week after treatment (p<0.05) (Fig. 1).

Blood lipid test results. Levels of TC, TG, LDL-C and HDL-C in treatment and model group were significantly higher than those in control group (p<0.05). After treatment, levels of TC, TG, LDL-C and HDL-C in treatment group gradually decreased (p<0.05) (Table I).

Liver function test results. Levels of AST, ALT and GGT in treatment and model group were significantly higher than those in control group (p<0.05). After treatment, levels of AST, ALT and GGT in rats of treatment group gradually decreased (p<0.05) (Table II).

HIF-1α test results. Relative HIF-1α expression level was 0.40±0.01 in control group and 1.30±0.30 in the model group. Relative HIF-1α expression level was 1.24±0.14 in treatment group at 12 h after model construction, 0.86±0.10 at 1 week after treatment and 0.41±0.03 at 3 weeks after treatment. Expression level of HIF-1α was significantly higher in model and treatment group than in control group at 12 h after model construction and 1 week after treatment (p<0.05). No significant differences in the expression level of HIF-1α were found between model and treatment group at 12 h after

Table I. Blood lipid test results of two groups of rats (mmol/l).

| | | Treatment group | | | |
Items	Control group	12 h after model construction	1 week after treatment	3 weeks after treatment	Model group
TC	0.32±0.24	4.48±1.27[a]	3.54±0.94[a,b]	1.92±0.76[a-c]	4.52±1.36[a-d]
TG	0.61±0.25	1.52±0.92[a]	1.14±0.46[a,b]	0.89±0.24[a-c]	1.61±0.87[a-d]
LDL-C	0.24±0.10	2.08±1.09[a]	1.52±0.86[a,b]	0.96±0.31[a-c]	2.04±1.02[a-d]
HDL-C	1.04±0.24	2.28±0.53[a]	1.83±0.47[a,b]	1.37±0.56[a-c]	2.20±0.41[a-d]

[a]$P<0.05$, compared with control group; [b]$p<0.05$, compared with 12 h after model construction; [c]$p<0.05$, compared with 1 week after treatment; [d]$p<0.05$, compared with 3 weeks after treatment. TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Table II. Liver function test results of two groups of rats (IU/l).

| | | Treatment group | | | |
Items	Control group	12 h after model construction	1 week after treatment	3 weeks after treatment	Model group
AST	18.25±4.51	36.34±6.27[a]	30.69±4.01[a,b]	24.36±5.83[a-c]	35.84±6.04[a-d]
ALT	24.43±4.92	37.42±8.22[a]	33.14±7.16[a,b]	28.77±6.04[a-c]	36.62±8.04[a-d]
GGT	27.15±6.27	42.52±9.81[a]	38.05±4.37[a,b]	34.53±6.98[a-c]	41.60±9.70[a-d]

[a]$P<0.05$, compared with control group; [b]$p<0.05$, compared with 12 h after model construction; [c]$p<0.05$, compared with 1 week after treatment; [d]$p<0.05$, compared with 3 weeks after treatment. AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl transferase.
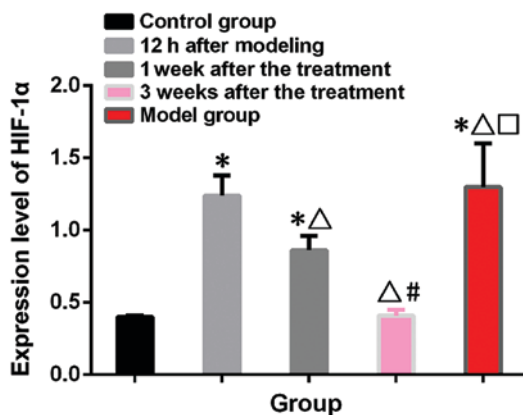

Figure 2. HIF-1α test results of two groups of rats. There was no significant difference between model and treatment group at 12 h after model construction. Expression level of HIF-1α was significantly higher in model and treatment group at 12 h after model construction and 1 week after treatment. In treatment group, expression level of HIF-1α decreased with the prolonged treatment, and no significant differences in expression level of HIF-1α were found between treatment and control group at 3 weeks after treatment. *$p<0.05$, compared with control group; △$p<0.05$, compared with model group at 12 h after model construction; #$p<0.05$, compared with 1 week after treatment; □$p<0.05$, compared with 3 weeks after treatment. HIF-1α, hypoxia-inducible factor-1α.

model construction ($p>0.05$). In treatment group, the expression level of HIF-1α decreased with the prolonged treatment, and no significant differences in expression level of HIF-1α were found between treatment and control group 3 weeks after treatment ($p>0.05$) (Fig. 2).

Discussion

T2DM can easily lead to damage of important organs in human body and has been proved to be a major cause of a variety of heart, brain, liver and eye diseases (12). At present, there is no clear conclusion on the pathogenesis of T2DM-induced fatty liver disease. Insulin resistance and oxidative stress lipid peroxidation is a major cause of inflammatory necrosis and fibrosis in the liver (13,14). HIF-1, a DNA-binding protein discovered by Semenza and Wang (15) in 1992, is composed of two subunits, α and β, and has been shown to have various immune responses in the human body through hypoxic-ischemic conditions (16,17). The role of HIF-1 in patients with T2DM complicated with fatty liver is unclear. In this study, rats T2DM and fatty liver models were induced by high-fat diet and treated with saxagliptin. The expression of HIF-1α was detected to examine the role of HIF-1α in T2DM and fatty liver.

In this study, compared with control group, the expression level of HIF-1α in model group was significantly higher than that in control group, and the expression levels of HIF-1α in rat models were decreased gradually with the prolonged saxagliptin treatment and reached the level of control group 3 weeks after treatment, indicating the involvement of HIF-1α in the onset and development of T2DM, which is consistent

with the findings of Nayak *et al* (18). The possible explanation is that HIF-1α promotes the transcription of VEGF under the condition of hypoxia, so as to induce the regeneration of liver cells in rats with fatty liver (19). Over-proliferation of cells accelerates the consumption of oxygen in rats, and the internal circulatory system cannot provide enough oxygen, which in turn induces the expression of HIF-1α, resulting in a vicious circle. HIF-1α stimulates the secretion of a large number of cellular stimulating factors, so as to exacerbate insulin secretion and reduce insulin sensitivity, thus contributing to the development of fatty liver (20). Saxagliptin can also effectively improve blood glucose and lipids and liver function by improving insulin resistance metabolic disorders (21). With the prolonged treatment, those indexes gradually decreased, indicating that saxagliptin can effectively improve the blood glucose, blood lipid and liver function. Insulin resistance induces the synthesis of free fatty acids in adipose tissue, which can result in elevation of GLP-1 in rats, leading to further deterioration of fatty liver (22). Overload of GLP-1 directly affects the normal metabolic function of the digestive system of rats and greatly reduces the absorption of nutrients. Saxagliptin increases the synthesis and metabolism of cyclic adenosine monophosphate and inhibits the secretion of glucagon, which can not only promote the proliferation and differentiation of β cells, but also activate the protective effect of GLP-1 receptor on liver of rats (23). Therefore, re-deterioration of liver cirrhosis in rats was suppressed. With saxagliptin, insulin in rats is effectively maintained and the activation of VEGF is inhibited, resulting in gradually decreased expression level of HIF-1α.

In this study, upregulated expression of HIF-1α was observed in diabetic rats combined with fatty liver and saxagliptin effectively inhibited the expression of HIF-1α. However, animal system may be different from human body. Therefore, clinical studies are needed to further confirm our conclusions.

In conclusion, HIF-1α is highly expressed in rats with T2DM and fatty liver. Saxagliptin can effectively improve the blood glucose, blood lipid and liver function, and reduce the expression level of HIF-1α protein.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

QZ was responsible for writing the manuscript and model construction. QX and XM analyzed and interpreted blood lipid results and blood glucose tests. JC performed liver function test. WY helped with HIF-1α test. YZ performed western blot analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Dahua Hospital of Xuhui District (Shanghai, China).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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