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Beneficial effects of liraglutide on peripheral blood vessels

Korisni efekti liraglutida na periferne krvne sudove

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Abstract

Background/Aim. Macroangiopathy is the major cause of death and disability in type 2 diabetic patients. Studies have shown that liraglutide, a glucagon-like peptide 1 (GLP-1) receptor agonist, can protect cardiovascular system by inhibiting chronic inflammation of diabetes. However, a study about the effects of liraglutide on peripheral blood vessels and peripheral blood leukocytes has not been reported yet. The aim of this study was to determine vasculoprotective effect, vascular protection and mechanism of action of liraglutide in addition to its hypoglycemic effect. **Methods.** A total of 60 hospitalized patients with type 2 diabetes were recruited from December 2013 to December 2014 at the First Affiliated Hospital of Dalian Medical University, PR China. Before the treatment with liraglutide, height and weight were measured to calculate body mass index (BMI). Blood urea nitrogen (BUN) and so on were detected. Homeostasis model assessment of insulin resistance (HOMA-IR) and islet β cell function (HOMA-β) were computed. After applying liraglutide for three months, all indexes were measured again. The effects of liraglutide on these indexes were analyzed by paired sample *t*-test. **Results.** After the treatment with liraglutide, values of glycosylated hemoglobin – HbA1c (8.46 ± 1.62 vs. 7.26 ± 1.40%) and 2h postprandial blood glucose – 2hPBG (11.95 vs. 9.6 mmol/L) decreased significantly ($p < 0.05$). Body weight (87.3 vs. 82.5 kg) and BMI (30.37 vs. 28.63 kg/m^2) decreased by 5.5% and 5.7%, respectively ($p < 0.05$). Also, levels of triglycerides (TG) (2.57 ± 1.54 vs. 1.81 ± 0.70 mmol/L) and LDL-cholesterol (2.92 ± 0.78 vs. 1.89 ± 0.66 mmol/L) reduced significantly ($p < 0.05$). Ankle-brachial index (ABI) decreased from 1.24 ± 0.10 to 1.14 ± 0.06 cm/s by 8%, while brachial-ankle pulse wave velocity (ba-PWV) decreased from 1,442.15 ± 196.26 to 1,316.85 ± 146.63 cm/s by 8.7%, and both differences were statistically significant ($p < 0.001$). **Conclusion.** Liraglutide, with a good hypoglycemic effect, can significantly reduce postprandial blood glucose and HbA1c, but cannot significantly improve fasting plasma glucose, insulin resistance and islet β cell function. It also considerably decreased body weight, BMI and TG. Liraglutide can significantly lower ba-PWV and ABI to protect peripheral blood vessels.

Key words:
ankle-brachial index; arterioles; blood vessels; body mass, index; capillaries; diabetes mellitus, type 2; leukocytes; lipids; liraglutide; venules.

Apstrakt

Uvod/Cilj. Makroangiopatija je glavni uzrok smrti i invalidnosti kod bolesnika sa dijabetesom tipa 2. Studije su pokazale da liraglutid, agonist *glucagon-like peptide* 1 (GLP-1) receptora, može zaštititi kardiovaskularni sistem inhibicijom hroničnog zapaljenja prouzrokovanog dijabetesom. Međutim, efekti liraglutida na periferne krvne sudove i leukocite u perifernoj krvi nisu opisani do sada. Cilj ovog rada bio je da se, pored hipoglikemijskog dejstva liraglutida, ispita i njegov vaskuloprotektivni efekat, kao i mehanizam tog dejstva. **Metode.** U studiju je bilo uključeno 60 bolesnika sa dijabetesom tipa 2 hospitalizovanih od decembra 2013. do decembra 2014. godine u Prvoj bolnici Medicinskog univerziteta Dalian iz Kine. Pre početka terapije liraglutidom, svim bolesnicima su izmerene visina i telesna masa da bi se izračunao indeks telesne mase (ITM). Takođe, određene su i vrednosti uree u krvi (BUN) i drugi biohemijski parametri. Izračunate su vrednosti HOMA-IR (*Homeostasis model assessment of insulin resistance*) i HOMA-β (*Homeostasis model assessment of islet β cell function*) indeksa. Posle tri meseca primene liraglutida, ponovo su određeni svi indeksi. Efekti liraglutida na te indekse analizirani su *t*-testom zavisnih uzoraka. **Rezultati.** Posle tromesečnog lečenja liraglutidom, vrednosti glikoziliranog hemoglobina A1c – HbA1c (8,46 ± 1,62% vs. 7,26 ± 1,40%) i nivoa glukoze u krvi 2 časa posle obroka (2hPBG) (11,95 mmol/L vs. 9,6 mmol/L) značajno su se smanjili ($p < 0,05$). Telesna masa (87,3 kg vs. 82,5 kg) i ITM (30,37 kg/m^2 vs. 28,63 kg/m^2) smanjili su se 5,5%, odnosno 5,7% ($p < 0,05$). Nivoi triglicerida (2,57 ± 1,54 mmol/L vs. 1,81 ± 0,70 mmol/L) i LDL-holesterola (2,92 ± 0,78 mmol/L vs. 1,89 ± 0,66 mmol/L) takođe su se značajno smanjili ($p < 0,05$). *Ankle-brachial index* (ABI) smanjio se sa 1,24 ± 0,10 cm/s na 1,14 ± 0,06 cm/s ili za 8%, a *brachial-ankle pulse wave velocity* (ba-PWV) sa 1 442,15 ± 196,26 cm/s na 1 316,85 ± 146,63 cm/s ili za 8,7%, što je u oba slučaja

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bilo statistički značajno smanjenje (p <0,001). **Zaključak**. Liraglutid, sa dobrim hipoglikemijskim efektom, može značajno smanjiti nivo glukoze u krvi nakon obroka i vrednost HbA1c, ali ne utiče značajno na nivo glukoze u plazmi natašte, insulinsku rezistenciju i funkciju β ćelija Langerhansovih ostrvaca pankreasa. Takođe, lek značajno smanjuje telesnu masu, ITM i nivo triglicerida kao i vrednosti ba-PWV i ABI, pokazatelje zaštite perifernih krvnih sudova.

Ključne reči:
brahijalni indeks gležnja; arteriole; krvni sudovi; telesna masa, indeks; kapilari; diabetes mellitus, tip 2; leukociti; lipidi; liraglutid; venule.

Introduction

The China Chronic Diseases and Risk Factors Surveillance in 2013 showed that the prevalence of diabetes mellitus (DM) in people aged 18 or older was 10.4% [1]. DM is an independent risk factor for cardiovascular and cerebrovascular diseases [2]. The key to the treatment of type 2 DM (T2DM) is long-term stable control of blood glucose to prevent or delay the occurrence and development of chronic complications of diabetes. Epidemiological studies have found that T2DM patients have a 2–4 times higher risk of developing myocardial infarction and stroke than normal people have [3, 4]. The prevention and treatment of diabetic macroangiopathy should be based on the control of blood sugar, blood pressure, blood lipids, body weight and other factors. The deposition of advanced glycosylation end products caused by persistent hyperglycemia, insulin resistance, hypertension and hyperlipidemia lead to an increase in oxidative stress, promotion of aggregation of blood mononuclear macrophages, and release of proinflammatory cytokines, that further damage vascular endothelium leading to atherosclerosis [5, 6].

Glucagon-like peptide 1 (GLP-1) receptor agonists are a newer generation of hypoglycemic drugs. In addition to controlling blood sugar, they have good effects on reducing body weight, improving insulin resistance, treating fatty liver and protecting cardiovascular system [7–9]. Liraglutide is a glucagon-like peptide 1 (GLP-1) analogue produced by yeast using genetic recombination [10]. In the Liraglutide Effect and Action in Diabetes: Evaluation of cardiovascular outcome Results (LEADER) study, liraglutide was confirmed to have the effects of lowering blood sugar and glycosylated hemoglobin (HbA1c), improving insulin resistance, protecting islet function, decreasing blood lipids and body weight, and reducing vasoconstriction pressure [11–18]. Liraglutide has been shown to protect cardiovascular function at both cellular models and animal models [19]. Liraglutide has also been proven to reduce cardiovascular events in diabetic patients during clinical trials and to protect cardiovascular system by managing cardiovascular risk factors [20].

Atherosclerosis is a systemic disease, which often involves multiple blood vessels such as carotid arteries, lower extremity arteries, and renal arteries in addition to coronary arteries and cerebral arteries [21]. Because the carotid and lower extremity arteries are superficial and easy to examine, they are often considered to be the windows of systemic atherosclerotic disease. Epidemiological investigations have shown that peripheral arteriosclerotic diseases seriously affect the prognosis of other cardiovascular and cerebrovascular diseases. With the advancement of inspection techniques, high-sensitivity noninvasive vascular examinations have been used in clinical practice. Pulse wave velocity (PWV) and ankle-brachial index (ABI) are two important evaluation indicators in these examinations. As an indicator of arterial stiffness, PWV can independently predict the risk of cardiovascular and cerebrovascular disease development and death [22]. ABI can be used for an early diagnosis of lower extremity obstructive disease [23]. These two examinations can evaluate vascular arteriosclerosis from both function and structure of the blood vessels. Arteriosclerosis often changes vascular function first and then it changes vascular structure. Increased PWV can occur in a variety of diseases associated with atherosclerosis, such as DM, high blood pressure, dyslipidemia and severe kidney disease. Depending on selected arteries, PWV can be divided into carotid-femoral PWV, carotid-radial PWV, carotid-brachial PWV, and brachial-ankle PWV (ba-PWV). The study found that ba-PWV was a better predictor of T2DM macrovascular complications than carotid PWV [24]. Ba-PWV of more than 1,400 cm/s usually suggests an increase in vascular stiffness. ABI is the ratio of systolic pressure of the ankle to systolic pressure of the arm, which reflects the degree of the openness of peripheral blood vessels. The normal value of ABI is 0.9–1.3. ABI of more than 0.9 often indicates peripheral obstructive vascular disease, while ABI that is less than 1.3 often points out arterial calcification and weakened vasoconstriction. A systematic retrospective study manifested that ABI could be used to predict the occurrence of cardiovascular diseases [25].

Inflammation and insulin resistance are thought to be the basis of atherosclerosis in T2DM [26]. Studies have shown that increased white blood cells can worsen insulin sensitivity and predict the development of DM [27]. Mononuclear cells in blood infiltrate into the intima and differentiate into macrophages. Infiltrating macrophages phagocytose oxidatively modified low-density lipoproteins and gradually get transformed into foam cells. This leads to the development of atherosclerosis. Adherence of mononuclear cells to vascular endothelial cells is considered to be one of the earliest events in the complex mechanism of atherosclerosis [28]. Inflammatory mediators associated with the pathogenesis include interleukin 6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor alpha (TNF-α). CRP is an inflammatory factor in the development and progression of atherosclerosis. Any high concentration of CRP is closely related to the occurrence of cardiovascular diseases. CRP can increase the expression of cell adhesion molecules and monocyte chemoattractant protein 1 (MCP-1), promote the production of matrix metalloproteinase 1 (MMP-1), activate the complement system to promote the production of arteriosclerosis, and reduce the expression of nitric oxide synthetase mRNA and bioactive activity of nitric oxide in endothelium [29].

Methods

Patients

The study included T2DM patients with poor glycemic control admitted to the Department of Endocrinology at the First Affiliated Hospital of Dalian Medical University from December 2013 to December 2014, aged between 18 and 70 years, with fasting C-peptide (FCP) > 1 ng/mL, HbA1c 7–11%. The T2DM diagnosis meets the diagnostic and classification criteria approved by the World Health Organization (WHO) in 1999.

Those patients who met any of the following criteria were excluded from the study: severe renal dysfunction, creatinine clearance (CCR) < 60 mL/min; abnormal liver function, alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 120 U/L combined with severe acute complications of DM and stress; infection and malignancy; severe history of cardiovascular and cerebrovascular disease in previous 3 months; pregnancy, preparing pregnancy and lactation period in women; various diseases with a significant effect on blood sugar; using drugs other than hypoglycemic drugs that affect blood sugar, such as hormones; a history of pancreatitis and a history or family history of medullary thyroid carcinoma.

Exit criteria were as follows: violation of inclusion criteria or compliance with exclusion criteria; serious adverse drug reactions; drug allergic reactions; loss of patients during follow-up.

Experimental drugs and administration methods

Liraglutide (6 mg/mL), produced by Danish Novo Nordisk, is subcutaneously injected once a day. The drug can be injected at any time without depending on meal time. The patients were recommended to get injections at the same time every day when it was the most convenient for them.

Those patients who met the inclusion criteria were given diabetic education and informed about the role and possible side effects of liraglutide. They filled in the informed consent form and were adjusted to the treatment plan.

General information

Age, sex and medical history of the enrolled patients were recorded in detail. Height and body weight were measured according to standard protocols to calculate body mass index (BMI) for each patient.

The patients were taken blood from in the early morning after 12 hours of overnight fasting. An automated biochemical analyzer measured urea nitrogen (BUN), creatinine (Cr), glycosylated hemoglobin A1c (HbA1c), fasting venous blood glucose (FPG), fasting C-peptide (FCP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), white blood cells (WBC), monocytes (M), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and CRP. Fingertip 2h posprandial blood glucose (2hPBG) was measured by the Roche blood glucose meter.

Parameter calculation formula

Parameter calculation formulas were as follows:

CCR = [(140-age) × body weight (kg)/0.818 × Cr (μmol/L)]; homeostasis model assessment of insulin resistance (HOMA-IR) = 1.5 + FPG (mmol/L) × FCP (pmol/L)/2,800; homeostasis model assessment of islet β cell function (HOMA-β) = 0.27 × FCP (pmol/L)/[FPG (pmol/L) - 3.5]; BMI = body weight (kg)/height^2 (m^2).

Experiment process

The temperature of the examination room was kept at 22–25 °C, and the other parameters were taken 5 minutes before the measurement. The patients took off their heavy clothing and removed the socks to reveal the heel. They were lying in the supine position on the bed, with the upper arm and ankle strapped for the electrocardiogaraphy (ECG) clip and heart sound map sensor. Heart sound map, heart rate value, left arm blood pressure, left arm pulse wave, right arm blood pressure value, right arm pulse wave, left ankle blood pressure value, left ankle pulse wave, right ankle blood pressure value, and right ankle pulse wave data were collected. The process allowed the acquisition to be completed, so the instrument automatically calculated ba-PWV and ABI after the cuffs were filled and deflated twice.

The enrolled patients recorded general information in detail and signed informed consent on the day of their admission. They were measured according to the above given indicators and the next day for noninvasive peripheral blood vessel testing. The starting dose of liraglutide was 0.6 mg once a day for subcutaneous injecting, and the patients were observed for adverse drug reactions. If there was no obvious adverse reaction after 3–7 days, the dose was increased to 1.2 mg once daily. If there was a significant gastrointestinal reaction after the drug addition, the dose could be reduced back to 0.6 mg/day, and the dose was increased after the low-dose administration time was extended. The dose was increased to 1.8 mg/day from 1.2 mg/day after a week for the patients with poor hypoglycemic effect.

Statistical analysis

The normality of continuous variables was verified by S-K test. Variables that conform to normal distribution are expressed as mean ± standard deviation (SD) and the difference between the before and after liraglutide treatment was analyzed by the paired *t*-test. The non-normal distribution variables were expressed by quartile, and the differences were compared by the nonparametric Wilcoxon test. Significant variables screened by univariate analysis were further analyzed by multiple regression analysis to prove independent factors. SPSS 25.0 software was used for statistical analysis. A double-tailed *p*-value less than 0.05 was considered statistically significant.

Results

In this study, 64 patients with type T2DM were initially enrolled. Four of them dropped out during the follow-up, so 60 patients were finally enrolled (Table 1). Among them, there were 31 males and 29 females. They were 46.85 ± 7.60 years old, with the disease history of 8 (3–10) years. Twenty one of them had complications and 10 had chronic complications of DM. Noninvasive angiography was performed in 13 cases. After the use of liraglutide, the patients had adverse reactions such as nausea and loss of appetite in varying degrees, but they were tolerable. Later on, the patients' nausea and other discomfort gradually disappeared. No patients were withdrawn because of severe gastrointestinal reactions. There were no allergies at the injection sites. One patient had a marked hypoglycemia due to liraglutide, but it was tackled after a prompt symptomatic treatment.

Table 1

Baseline data for the patients

Parameter	mean ± SD/quartile	(minimum, maximum)
Gender (M/F), n	31/29	
Age△ (years)	46.85 ± 7.60	(32, 66)
History# (years)	8 (3~10)	(0.038, 17)
Biochemistry analysis		
ALT △(U/L)	39.42 ± 18.93	(7, 95)
AST# (U/L)	21 (17, 31)	(9, 64)
Urea# (μmol/L)	5.69 (5.21~6.51)	(3.34, 8.85)
Cr△ (μmol/L)	60.22 ± 13.26	(32, 98)
CCR△ (mL/min)	178.46 ± 48.34	(81.11, 319.53)
Comorbidity, n	21	-
Chronic complications, n	10	-
Side effects, n		
gastric reaction	25	-
hypoglycemia	1	-
allergic reactions	0	-

△ Variables are shown as mean ± standard deviation (SD) if the variable was conformed to normal distribution by S-K test; # Variables are shown as quartile 50% (25%~75%) if the variable was not conformed to normal distribution by S-K test; M – male; F – female; ALT – alanine aminotransferense; AST – aspartat aminotransferase; Cr – creatinine; CCR – creatinine clearance.

The data about diabetic blood lipid inflammation and vascular measurements in patients before and after liraglutide administration are given in Table 2.

After the 3-month treatment with liraglutide, the levels of HbA1c and 2hPBG decreased significantly ($p < 0.05$), indicating the notable hypoglycemic effect of liraglutide. Although FPG and FCP decreased in varying degrees with no significant difference, HOMA-IR and HOMA-β showed no distinct difference, suggesting the insignificant effects on improving insulin resistance and islet function.

Body weight decreased by 5.5% from 87.3 (80–101) kg to 82.5 (76–93) kg, while BMI decreased by 5.7% from 30.37 (29.43–31.83) kg/m^2 to 28.63 (27.78–30.80) kg/m^2, and both differences were statistically significant ($p < 0.001$). It can be concluded that liraglutide can considerably reduce body weight and BMI.

TG decreased from 2.57 ± 1.54 mmol/L to 1.81 ± 0.70 mmol/L with the decrease of 29.6%. LDL-C decreased by 35.3% from 2.92 ± 0.78 mmol/L to 1.89 ± 0.66 mmol/L and both differences were statistically noteworthy ($p < 0.001$). TC and HDL-C decreased by 12% and 4.8%, respectively, yet without any significant difference ($p > 0.05$), suggesting that liraglutide may effectively bring down the levels of TG and LDL-C.

After the liraglutide treatment, M, CRP and WBC all decreased in varying degrees, but the differences were not statistically relevant.

ABI and ba-PWV are important indexes reflecting the level of peripheral vascular stiffness. Three months after the treatment, ABI decreased from 1.24 ± 0.10 cm/s to 1.14 ± 0.06 cm/s by 8%, while ba-PWV decreased from 1,442.15 ± 196.26 cm/s to 1,316.85 ± 146.63 cm/s by 8.7%, thus revealing that liraglutide may improve peripheral vascular stiffness effectively.

Multivariate regression analysis for ba-PWV is shown in Table 3.

Multiple regression analysis showed that increment of ABI – in-ABI (increment = difference between values of a variable: 3rd month vs. baseline), in-HbA1c and in-TG were independent risk factors for in-ba-PWV. The contributions to ba-PWV are in-ABI, in-HbA1c and in-TG in turn. According to the correlation coefficient, the following regression equation is constituted:

$$\text{in-ba-PWV} = 40.975 + 524.447 \times \text{in-ABI} + 16.287 \times \text{in-HbA1c} + 18.79 \times \text{in-TG}$$

Table 2

Comparing diabetic, blood lipid, inflammatory and vascular stiffness data in patients before and after liraglutide administration

	Baseline	3rd month	*t*	*p*
HbA1c△ (%)	8.46 ± 1.62	7.26 ± 1.40	5.234**	< 0.001
FPG# (mmol/L)	8.3 (7.24~8.84)	7.46 (6.52~8.67)	-1.612	0.107
2hPBG# (mmol/L)	11.95 (10.15~13.4)	9.6 (8.425~11.05)	-5.913**	< 0.001
FCP△ (ng/mL)	3.24 ± 1.37	3.05 ± 0.77	1.198	0.236
HOMA-IR△	4.60 ± 1.39	4.25 ± 0.87	1.813	0.075
HOMA-β△	62.47 ± 33.02	69.78 ± 39.09	-1.711	0.092
BW# (kg)	87.3 (80~101)	82.5 (76~93)	-5.974**	< 0.001
BMI# (kg/m^2)	30.37 (29.43~31.83)	28.63 (27.78~30.80)	-5.865**	< 0.001
TC# (mmol/L)	5.09 (4.03~5.67)	4.48 (3.74~5.36)	-1.612	0.107
TG△ (mmol/L)	2.57 ± 1.54	1.81 ± 0.70	4.083**	< 0.001
LDL-C△ (mmol/L)	2.92 ± 0.78	1.89 ± 0.66	18.936**	< 0.001
HDL-C△ (mmol/L)	1.24 ± 0.24	1.18 ± 0.24	1.614	0.112
M# (× 10^9/L)	0.43 (0.38~0.55)	0.43 (0.34~0.54)	-1.937	0.053
WBC△ (× 10^9/L)	7.03 ± 1.32	6.88 ± 1.39	0.615	0.541
CRP△ (mg/dL)	5.38 ± 2.45	5.16 ± 2.45	1.863	0.067
ABI△ (cm/s)	1.24 ± 0.10	1.14 ± 0.06	7.941**	< 0.001
ba-PWV△ (cm/s)	1,442.15 ± 196.26	1,316.85 ± 146.63	8.599**	< 0.001

HbA1c – glycosylated hemoglobin; FPG – fasting venous blood glucose; PBG – postprandial blood glucose; FCP –fasting C-peptides; HOMA-IR – homeostasis model assessment of insulin resistance; HOMA-β – homeostasis model assessment of β cell function; BW – body weight; BMI – body mass index; TC – total cholesterol; TG – triglycerides; LDL-C – low-density lipoprotein cholesterol; HDL-C – high-density lipoprotein cholesterol; M – monocytes; WBC – white blood cells; CRP – C-reactive protein; ABI – ankle-brachial index; ba-PWV – brachial-ankle pulse wave velocity.

△ Variables are shown as mean ± standard deviation (SD) and paired *t*-test was carried out if the variable was conformed to normal distribution by S-K test; # Variables are shown as quartile 50% (25%~75%) and paired nonparametric Wilcoxon test was carried out if the variable was not conformed to normal distribution by S-K test; * $p < 0.05$; ** $p < 0.001$.

Table 3

Multivariate Regression Analysis for increment of ba-PWV (3rd month vs. baseline)

Parameter	Unstandardized		Standardized β	*t*	*p*	Collinearity	
	β	SD				tolerance	VIF
Constant	40.975	22.014		1.861	0.068		
in-ABI (cm/s)	524.447	141.401	0.437	3.709	< 0.001	0.919	1.088
in-HbA1c (%)	16.287	7.227	0.256	2.254	0.028	0.986	1.015
in-TG (mmol/L)	18.790	9.181	0.240	2.047	0.045	0.929	1.076

ABI – ankle-brachial index; HbA1c – glycosylated hemoglobin; TG – triglycerides; SD – standard deviation; In – increment (difference between values of a variable: 3rd month vs. baseline); VIF – variance inflation factor.

Discussion

Macroangiopathy is one of serious complications of T2DM. Prevention of the occurrence and development of macroangiopathy requires comprehensive control. Therefore, new hypoglycemic drugs are particularly important for the role of diabetic macroangiopathy. This study observed 60 cases, including 13 cases of noninvasive vascular examination. The comparison between the before and after use of liraglutide showed that liraglutide could not only effectively reduce postprandial blood glucose, HbA1c, body weight, BMI, TG, but it could also reduce ba-PWV and ABI.

HbA1c guides diabetes treatment, shows blood sugar control levels, adjusts hypoglycemic regimens, influences the quality of care and predicts diabetes complications. Foreign studies have shown that HbAlc was closely related to diabetes complicated by cardiovascular and cerebrovascular diseases [30]. The UK Prospective Diabetes Study (UKPDS) confirmed that for every 1% decrease in HbA1c, the risk of any endpoint associated with DM was reduced by 21% and the risk of developing myocardial infarction was reduced by 18%. In Chinese Type 2 Diabetes Guidelines (2017), HbA1c control targets should be < 6.5% for patients with T2DM who have a shorter course, no complications and a longer life expectancy without a cardiovascular disease. In this research,

HbA1c decreased significantly from 8.46 ± 1.62% to 7.26 ± 1.40%, which was consistent with the results of the LEADER studies. The LEADER series of studies showed that liraglutide significantly reduced HbA1c in patients with T2DM and was superior to glimepiride and rosiglitazone [13–18]. However, the average level of HbA1c in some patients in this study still did not meet the standard (HbA1c < 7%) considering the higher levels of HbA1c before the application of liraglutide, the shorter follow-up time, and the smaller dose of liraglutide. 2hPBG decreased significantly from 11.94 ± 3.17 mmol/L before the treatment to 9.63 ± 1.53 mmol/L ($p < 0.05$) 3 month later, but there was no significant difference between the before and after FPG ($p > 0.05$), suggesting that liraglutide can effectively reduce postprandial blood glucose, but it can only limitedly control fasting blood glucose. GLP-1 receptor agonists cause the hypoglycemic effect by delaying gastric emptying, promoting insulin secretion, inhibiting glucagon and somatostatin secretion, so this drug has an advantage in controlling postprandial blood glucose [31, 32].

Progressive failure of islet function and insulin resistance is considered to be the main pathological basis of T2DM. Long-term hyperglycemia and insulin resistance can cause atherosclerosis caused by the damage of vascular endothelium. Insulin resistance index can be expressed by HOMA-IR. It is generally believed that HOMA-IR increases with the increase of insulin resistance. HOMA-IR is a HOMA-IR formula fitted with C-peptide. Studies have confirmed that this formula could also be used to assess an individual's insulin resistance [33]. HOMA-IR and HOMA-β were calculated using this formula in our study. HOMA-IR and FCP decreased, while HOMA-β increased, but there was no statistical significance ($p > 0.05$). These results were different from other studies. The LEADER-2 study showed that islet function was assessed by the ratio of proinsulin to insulin, and liraglutide improved islet beta cell function compared to glimepiride [15]. In the LEADER-3 study, HOMA-IR evaluated insulin resistance, and liraglutide significantly improved insulin resistance in comparison with glimepiride [14]. The LEADER-4 studies have shown that liraglutide significantly increased islet function if compared with gliclazide, on the basis of HOME-β and proinsulin-to-insulin ratios evaluating islet beta cells function [16]. Moreover, liraglutide had the effect of reducing body weight, and the decrease in body weight also indirectly improved insulin resistance. In this study, the negative results of liraglutide for insulin resistance and islet function improvement might be related to fewer cases and shorter observation time.

T2DM is often accompanied by obesity. DM accompanied by obesity increases the risk of cardiovascular diseases. Current hypoglycemic drugs (such as insulin, sulfonylureas, thiazolidinediones) can cause an increase in body weight and GLP-1 receptor agonists inhibit food intake by directly acting on the hypothalamus. Weight loss is achieved by inhibiting gastric emptying through the autonomic nervous system. Liraglutide was confirmed not to increase body weight in phase II clinical trial and showed a weight-reducing effect in phase III clinical trial. Liraglutide 1.8 mg/day, individually or in combination with other hypoglycemic agents, had a significant weight-reducing effect in the LEADER-1 to 5 series of studies [12–17]. It was found that liraglutide could reduce visceral fat by using computed tomography to analyze body composition in the LEADER-2 and LEADER-3 studies. Body weight decreased significantly from 87.3 (80–101) kg to 82.5 (76–93) kg and BMI decreased significantly from 30.37 (29.43–31.83) kg/m^2 to 28.63 (27.78–30.80) kg/m^2 ($p < 0.05$) in this study, which again confirmed that liraglutide could reduce patients' body weight and BMI. Liraglutide was confirmed to have the effect on reducing visceral fat in another study conducted in parallel with this.

Blood lipids are one of cardiovascular risk factors, including TC, TG, HDL-C, LDL-C and free fatty acids (FFAs). GLP-1 receptor agonists can directly act, inhibit gastric emptying, promote insulin secretion and increase the clearance of chylomicrons, and promote and enhance intestinal lipoprotein catabolism. Liraglutide reduces the levels of TC, LDL-C, TG, HDL-C and FFAs in the LEADER-4 study [13–14]. In this study, TG and LDL-C decreased significantly after the treatment with liraglutide, while there was no significant difference between TC and HDL-C that decreased after using liraglutide.

T2DM is often accompanied by systemic complications that show no early symptoms and are difficult to detect through symptoms. Early diagnosis, early intervention and prevention of progression of diabetes complications are particularly important in the management of DM. Diabetic macroangiopathy is the leading cause of death and disability in T2DM, so it is necessary to establish an early diagnosis of macroangiopathy. Arteriosclerosis is the main pathophysiological basis of diabetic macroangiopathy. Arterial stiffness is related to arteriosclerosis and can be used to predict cardiovascular and cerebrovascular diseases. ABI and ba-PWV are indicators of atherosclerosis. Ba-PWV is a vascular functional test for the detection of early atherosclerosis. ABI is a structural examination of blood vessels that can be used to understand the openness of blood vessels in the lower extremities and whether there is occlusion. A large number of clinical trials have demonstrated that liraglutide can reduce the occurrence of cardiovascular adverse events and protect cardiovascular function. However, the impact of liraglutide on peripheral blood vessels has not been reported yet In this study, the effects of liraglutide on ba-PWV were statistically significant ($p < 0.05$), indicating that liraglutide reduced peripheral vascular stiffness and protected peripheral blood vessels. ABI > 1.3 suggests vascular calcification and reduced vasoconstriction, while ABI < 0.9 indicates arterial stenosis. In this group study, ABI declined significantly from 1.24 ± 0.10 cm/s to 1.14 ± 0.06 cm/s after the drug administration, which suggested the vasoconstriction was better than before the treatment. The decrease of ABI value also predicted the improvement of arteriosclerosis by liraglutide treatment.

The mechanism of liraglutide in improving arteriosclerosis in patients with T2DM is not fully understood and

may be the result of a combination of inhibition of inflammation and non-inflammation. Noyan-Ashraf et al. [34] fed C57BI6 mice for 32 weeks on a high-fat diet (45% of calories from fat) and a normal diet, and randomly divided the two groups into two subsegments during the last week of feeding. In the subgroups, liraglutide (30 μg/kg, 2 times/day) and placebo were injected respectively [34]. It was found that high-fat diet induced an increase in serum TNF-α, while treatment with liraglutide for 1 week was effective in reducing high-fat diet-induced elevation of TNF-α. It was detected that liraglutide could reduce expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) in culture of human vascular endothelial cells *in vitro* [35]. In human umbilical vein endothelial cells (HUVEC) cultured *in vitro*, it was discovered that liraglutide intervention induced the synthesis of nitric oxide (NO), and the concentration of NO increased in a dose-dependent manner with liraglutide. At the same time, it was observed that 1 μg/mL liraglutide could enhance the activity of endothelial nitric oxide synthase (eNOS) and restore the expression of eNOS at mRNA level induced by cytokines. The studies in human umbilical vein endothelial cells (HCAEC) have also unearthed that liraglutide could increase eNOS phosphorylation and NO production by activating the AMP-activated protein kinase (AMPK) pathway [35]. Torres et al. [36] have confirmed that liraglutide could increase the release of calcium ions from endoplasmic network in vascular smooth muscle cells and increase mitochondrial calcium ion uptake through mitochondrial fusion protein-2, a key factor in mitochondrial-endoplasmic reticulum coupling, to enhance mitochondrial activity. Liraglutide can reduce endoplasmic reticulum stress induced by high glucose in HUVEC and also inhibit the expression of p53 up-regulated modulator of apoptosis. Liraglutide can prevent endoplasmic reticulum-dependent apoptosis of vascular endothelial cells through mitochondrial modulation.

The concept that atherosclerosis is an immune-mediated inflammatory lesion has been widely accepted. In this study, inflammatory related indicators, such as WBC, M and CRP, were detected in the vascular protection mechanism. Increased peripheral blood leukocytes can be used to predict the development of DM. M and macrophages are prototype cells of innate immune system and they are present in various stages of atherosclerosis. Studies on apoE-deficient mice have revealed that liraglutide significantly reduced the area of aortic atherosclerosis by inhibiting M/macrophage aggregation and reducing foam cell formation to prevent the occurrence of atherosclerosis [37]. The clinical study of liraglutide on peripheral blood leukocytes and M has not been reported yet. Since the aim of this research was to discuss the effects of liraglutide on WBC and M in peripheral blood vessels so as to explore the possible mechanism of liraglutide on vascular protection, the analysis of WBC and M before and after the treatment showed that WBC dropped from 7.03 ± 1.32×10^9/L to 6.88 ± 1.39×10^9/L, and M went down from 0.43 (0.38–0.55)×10^9/L to 0.43 (0.34–0.54)×10^9/L, but the differences were not statistically significant. Therefore, the examination of the impact of liraglutide on WBC and M in peripheral blood vessels may require a larger sample size and longer follow-up studies. CRP is an important marker of inflammation and studies have shown that GLP-1 receptor agonists can reduce the inflammatory response in diabetic patients and reduce the level of CRP in the body. This study found that there was no significant difference in CRP before and after the treatment with liraglutide, which was also inconsistent with the results of other studies. Van Raalte et al. [38] divided 69 T2DM patients with poor glycemic control treated by metformin into two groups, each of those receiving exenatide and insulin glargine respectively for 1 and 3 years. The results of the study showed that exenatide reduced significantly serum high-sensitivity CRP when compared with glargine. A meta-analysis also suggested that liraglutide significantly reduced serum CRP levels [39]. The negative results of CRP in this study may be related to fewer cases and large dispersion before the treatment. Hence, the effect of liraglutide on chronic inflammation in T2DM patients still requires further large-scale studies.

There were three limitations in this study. Firstly, the sample size included in this study was rather small. Secondly, no control group was used for comparative study results. Thirdly, follow-up time was short.

Conclusion

This study provides a new clinical basis for the beneficial effects of liraglutide on peripheral blood vessels. Liraglutide protects peripheral blood vessels in addition to its lowering blood sugar, reducing body weight and lowering blood lipids. Therefore, the results of this study may be the most beneficial to the treatment of patients with diabetic macroangiopathy or risk factors for diabetic macroangiopathy.

Liraglutide, with a good hypoglycemic effect, can significantly reduce postprandial blood glucose and HbA1c, but cannot considerably improve fasting plasma glucose, insulin resistance and islet function. It also significantly decreased body weight, BMI and TG. Liraglutide can significantly lower ba-PWV and ABI to protect peripheral blood vessels, but its effects on peripheral blood leukocytes, M and CRP need further research that may benefit from expanding the sample size and prolonging the treatment time.

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