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GERANIIN PREVENTS DIABETIC INDUCED BONE LOSS IN LIRAGLUTIDE TREATED DIABETIC RATS

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ABSTRACT

Liraglutide, a glucagon-like peptide-1 receptor agonist, is an anti-diabetic drug that has been linked to a lower incidence of fracture in diabetics. Rats with streptozotocin (STZ)-induced diabetes were given liraglutide and/or geraniin for eight weeks in this study. BMD (Bone mineral density) of the femur and lumbar vertebrae was assessed using dual-energy X-ray absorptiometry at the end of the trial (DXA). Serum glucose and glycosylated haemoglobin serum were also tested. Both alone and in combination, liraglutide and geraniin significantly reduced elevated blood glucose levels. Liraglutide treatment significantly lowered HBA1C levels when compared to the positive control. Geraniin and liraglutide together significantly lowered blood glucose and HBA1C levels. In the femur and lumbar vertebrae, liraglutide had little effect on BMD, where as geraniin treatment greatly improved these results. In conclusion, the findings imply that geraniin reduces STZ-induced bone degeneration in rats. These preliminary data suggest that geraniin may have a favourable effect on the bone health of diabetes postmenopausal women.

KEYWORDS

Bone mineral density, Bone health and Diabetic rats.

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INTRODUCTION

Diabetes mellitus is a common metabolic illness caused by insulin shortage or insulin resistance and it is characterised by hyperglycemia and a variety of chronic consequences. In patients with type 1 diabetes mellitus, a subtype of diabetes caused by the inability of pancreatic beta-cells to release insulin, clinical data revealed that bone mineral density

(BMD) was below normal[1]. One of the key causes that leads to bone fragility and an increased risk of fractures in diabetic patients is a decrease in BMD and reduced bone structure caused by diabetes, which has a complicated aetiology[1,2].

Osteoporosis is a metabolic bone disease characterised by bone loss and microarchitecture degradation. It primarily affects the elderly, particularly postmenopausal women and is associated with hip and vertebral fractures[3,4]. Under healthy conditions, bone remodelling, which refers to continuous cycles of bone resorption by osteoclasts and bone creation by osteoblasts, is in a homeostatic state[5]. Estrogen shortage causes faulty bone production and strong bone resorption in postmenopausal osteoporosis, resulting in gradual bone loss and an increased risk of fracture[5]. Diabetic postmenopausal women with a high risk of fracture have received special attention in terms of bone health care and fracture prevention[6-9].

Liraglutide, a glucagon-like peptide-1 (GLP-1) receptor agonist (GLP-1 RA), is a new anti-diabetic medicine that works by mimicking endogenous GLP-1 and increasing insulin secretion[10]. In diabetic individuals taking liraglutide medication, a meta-analysis of fracture incidence found a lower risk of incident fractures[11]. Liraglutide also has an anabolic bone impact in obese women who have lost weight[12]. In diabetic rodents, additional animal experiments revealed that liraglutide could prevent bone loss and slow down rapid bone degeneration[13,14]. Furthermore, in non-diabetic osteoporotic mouse models with ovariectomy (OVX), liraglutide improved bone mass and morphology[15,16]. Recent research has shown that geraniin can help with bone development, resorption and microstructure alterations[17].

Geraniin's potential bone-protective action in diabetic rats co-treated with liraglutide has yet to be investigated. The effects of liraglutide on BMD in a rat model of streptozotocin (STZ)-induced diabetes were investigated in this study.

MATERIAL AND METHODS[18,19]

Animals

The animals were acclimatised to the laboratory environment for 14 days. The treatment was carried out in accordance with the consent of King Khalid University's animal ethics committee and the National Institute of Health's guidelines for the care and use of laboratory animals in the United States (NIH Publication No. 85-23, revised 1996).

Induction of diabetes

To induce diabetes in rats, the pancreatic-cell toxin streptozotocin (STZ) (Sigma Chemical Co., freshly dissolved in sterile saline, 0.9 percent) was given intraperitoneally at a dose of 65mg/kg body weight. In the control group, all of the rats were given the same amount of vehicle. STZ was weighed separately for each animal, solubilized with 0.1ml of freshly prepared cold Na-citrate buffered (NaB-0.1 M, pH 4.5) and administered within 5 minutes to minimise deterioration. The volume of STZ injection was calculated to be 1.0ml/kg.

Rats were administered a 5% glucose solution for 48 hours after receiving STZ to counteract the drug's strong acute hypoglycemia effect. Three days following STZ injection, blood was taken from the tail vein and analysed for blood glucose using a glucometer (Aqua-Check, Roche). Animals having fasting blood glucose levels (BGLs) more than 250mg/dL were classified as diabetic. Group 1 (Non-Diabetic control), Group 2 (Diabetic control) and Group 3 (Geraniin 40mg/kg body weight), Group 4 (Liraglutide 150µg/kg bid s.c) and Group 5 (Liraglutide 150µg/kg bid s.c + Geraniin 40mg/kg body weight) were each divided into five groups of six rats. To establish the animals' hyperglycemic status, blood glucose levels were monitored once a week for the duration of the trial using a Roche Accu-Chek advantage glucometer.

Determination of fasting blood glucose

The study did not include the animals who did not develop blood glucose levels greater than 250mg/dL. The rats administered saline instead of streptozotocin in the control group (n=6) had normal blood glucose levels (≈120mg/dl). Blood samples were obtained from the rats' tail veins to test blood glucose levels using a glucometer after they had been fasted for 12-

14 hours. After the rats' tails have been washed with 70% (v/v) ethanol, blood will be drawn with a 1-ml needle, placed on a glucose strip and quantified with a glucometer.

Determination of intra-peritoneal glucose tolerance test

All of the rats were fasted for 12-14 hours before blood was taken from the tail vein as a baseline. The rats were subsequently given 2g/kg body weight (BW) of a 40% (w/v) glucose solution intraperitoneally. Blood will be collected from the tail vein and analysed for blood glucose using a glucometer after 30, 60, 90 and 120 minutes after glucose therapy. Fasting blood sugar readings of less than 250mg/dl were used to diagnose diabetes in these rats.

Determination of hemoglobin A1c

After blood samples from the tail vein are obtained and dropped on a test cartridge, haemoglobin A1c (HbA1c) will be analysed using a Clover A1cTM Self Analyzer. The Clover A1cTM Self Analyzer's LCD screen will show the percentage of HbA1c in the blood sample.

Bone Mineral Density Measurement

The BMD of the left femur and lumbar vertebrae (L1-L4) of rats was assessed using a dual energy X-ray absorptiometry (DEXA) scanning equipment after blood was collected (Lunar, WI, USA).

RESULTS AND DISCUSSION

The positive control group's (STZ) glucose profiles declined over time (Table No.1). However, both alone and in combination, liraglutide and geraniin have been shown to protect against diabetes development.

Table No.2 shows that HBA1C levels were greater in the positive control group than in the normal control group (p 0.05). Liraglutide and geraniin, alone and in combination, were observed to lower HBA1C levels in comparison to the positive control group, showing that geraniin had a beneficial effect.

The findings of bone mineral density study revealed that diabetic rats had lower lumbar (L1-L4) and femoral bone mineral density (BMD), which was recovered by Liraglutide and geraniin alone and in combination treatment (p 0.05). The BMD of the positive group and the other treatment groups differed significantly (Table No.3). These findings imply that geraniin may be able to protect bones from the effects of anti-diabetic medications.

Statistical analysis

The results must be expressed in terms of mean and standard deviation (SD). The data from different groups will be analysed statistically using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test. A p value of less than 0.05 is considered statistically significant.

Discussion

GLP-1 is an incretin hormone that is produced and secreted by gut L cells in response to meal consumption. It increases pancreatic beta-cell insulin release while suppressing alpha-cell glucagon secretion[20]. Because GLP-1 is rapidly destroyed in the body, stable GLP-1 RAs are being developed as a novel class of anti-diabetic medicines that imitate incretin functions[10]. Since the discovery of functional GLP-1 receptors on osteoblasts[21], the potential therapeutic effects of GLP-1 RAs have been studied in diabetic and non-diabetic mice[11,13,15]. In diabetic rodents, liraglutide treatment has been shown to increase BMD, enhance bone microarchitecture and restore mechanical characteristics of the bone[13,14] and it has also been shown to have favourable effects on the bone in non-diabetic osteoporotic animals[15,16] Literature back up liraglutide's bone-protective properties and give preliminary evidence for the drug's prospective effects on the bone health of postmenopausal diabetes women. Liraglutide treatment has been demonstrated to increase BMD, improve bone microarchitecture and restore mechanical features of the bone in diabetic rodents[13,14], as well as having beneficial effects on the bone in non-diabetic osteoporotic animals[15,16]. In this study, we used a rat model of osteoporosis caused by both diabetes and OVX and we discovered that long-term liraglutide treatment improved the defects in the bone of severely osteoporotic rats. Our findings support liraglutide's bone-protective characteristics and provide preliminary evidence for the drug's future benefits on postmenopausal diabetes women's bone health.

Table No.1: Effect of Geraniin in combination with Liraglutide on Fasting blood glucose level

S.No	Treatment Group	Dose	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56
1	Normal Control	5mL/kg	75.22 ±3.2	74.32 ±2.3	76.81 ±3.5	78.40 ±1.7	79.30 ±1.5	80.46 ±1.9	82.40 ±1.05	83.40 ±1.02	84.40 ±1.12
2	Positive Control	65mg/kg	261.54 ±10.2*	296.35 ±9.8*	314.21 ±12.62*	336.72 ±9.6*	351.72 ±8.4*	375.72 ±11.5*	398.72 ±10.5*	412.72 ±10.2*	435.72 ±9.6*
3	Geraniin	40mg/kg	266.33 ±7.3	286.25 ±9.4*	291.22 ±7.8*	296.28 ±8.2*	304.35 ±8.8*	307.35 ±9.8*	310.35 ±10.2*	320.35 ±9.2*	330.35 ±9.7*
4	Liraglutide	150µg/kg bid s.c.	243.32 ±7.3	235.23 ±9.4*	215.22 ±7.8*	210.24 ±8.2*	180.32 ±8.8*	150.35 ±9.8*	126.32 ±10.2*	101.33 ±9.2*	90.35 ±9.7*
5	Liraglutide +Geraniin	150µg/kg bid s.c.+ 40mg/kg	238.33 ±7.6*	217.24 ±8.4*	195.22 ±7.6*	176.26 ±7.2*	165.35 ±8.4*	130.39 ±8.8*	100.33 ±10.4*	98.35 ±9.6*	84.35 ±8.7*

Values are expressed as mean ± standard error of the mean (n=6)
*P<0.001 compared with normal control.

Table No.2: Effect of Geraniin in combination with Liraglutide on Glycoslyted Haemoglobin (HBA1C)

S.No	Treatment Group	Day 28
1	Normal Control	5.42±0.14
2	Positive Control	5.80±0.06*
3	Geraniin	5.68±0.03*
4	Liraglutide	5.43±0.12*
5	Liraglutide +Geraniin	5.44±0.13*

Values are expressed as mean ± standard error of the mean (n=6)
*P<0.001 compared with normal control.

Table No.3: Effect of Geraniin in combination with Liraglutide on the bone mineral density of the lumbar vertebrae and femur bone

S.No	Treatment Group	Bone Mineral density (mg/cm3)	
		Lumbar Vertebrae	Femur
1	Normal Control	178 ± 2.2	220 ± 2.5
2	Positive Control	78 ± 2.6*	100 ± 2.3*
3	Geraniin	158 ± 1.5*	200 ± 1.7*
4	Liraglutide	120 ± 2.2*	135 ± 2.5*
5	Liraglutide +Geraniin	168 ± 2.5*	217 ± 2.6*

Values are expressed as mean ± standard error of the mean (n=6)
*P<0.001 compared with normal control.

CONCLUSION

Finally, the current work shows that liraglutide and geraniin have a bone-preserving effect in a rat STZ-induced model. Our findings imply that liraglutide and geraniin may have a synergistic effect in maintaining bone health. This hypothesis, however, will need to be tested in future clinical trials.

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CONFLICTS OF INTEREST

"According to the authors, they have no competing interests. The funders had no role in the study's design, data collection, analysis, or interpretation, manuscript writing, or publication decision".

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