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CANAGLIFLOZIN INDUCED BONE LOSS IN DIABETIC RATS AND STRATEGY TO PREVENT IT

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ABSTRACT

Given that type 2 diabetes mellitus is a widespread metabolic condition with unfavourable effects on bone metabolism, the effect of anti-diabetic drugs on bone metabolism has gotten a lot of attention. Sodium-glucose co-transporter 2 (SGLT2) inhibitors (canagliflozin) are new anti-diabetic drugs that block glucose resorption in the kidney's proximal convoluted tubules, resulting in increased urine glucose excretion and lower blood glucose levels. The superiority of SGLT2 inhibitors is demonstrated in the reduction of glucose levels independent of insulin secretion, the reduction of hypoglycemia risk and the improvement of cardiovascular outcomes. SGLT2 inhibitors have been linked to genital mycotic infections, dehydration, orthostatic hypotension and ketoacidosis, as well as an elevated risk of acute renal injury. Furthermore, the impact of SGLT2 inhibitors on bone metabolism and fracture risk has been extensively studied. The goal of this study was to see if geraniin could help prevent canagliflozin-induced bone loss. For diabetes induction, streptozotocin was used. For eight weeks, diabetic rats were given either canagliflozin (40mg/kg) or geraniin (40mg/kg) alone or in combination. At the end of the experiment, BMD of the femur and lumbar vertebrae was measured by dual-energy X-ray absorptiometry (DXA). Glycoslyted Haemoglobin serum and serum glucose were also examined. Canagliflozin and geraniin, both alone and in combination, dramatically lowered high blood glucose levels. When compared to the positive control, canagliflozin therapy dramatically reduced HBA1C levels. The combination of geraniin and canagliflozin reduced blood glucose and HBA1C levels considerably. Canagliflozin had negative effects on BMD in the femur and lumbar vertebrae, while geraniin therapy significantly improved these effects. This study suggests that geraniin supplementation in diabetic patients using canagliflozin could be an appropriate method for reducing canagliflozin-induced bone loss.

KEYWORDS

Diabetic rats, SGLT2 and Canagliflozin.

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INTRODUCTON

Antihyperglycemic drugs (AHAs) that inhibit sodium glucose co-transporter 2 (SGLT2) have been developed to treat type 2 diabetes mellitus (T2DM)[1,2]. SGLT2 inhibitors, unlike other AHAs, decrease blood sugar by an insulin-independent mechanism that targets the kidney to enhance urine glucose excretion (UGE)[1-3]. Type 2 diabetes

mellitus (T2DM) alters bone metabolism and raises the risk of fracture[4,5]. Patients with T2DM are more likely to suffer hip fractures, which are the most severe of all osteoporotic fractures, as well as limb fractures such leg or ankle fractures[6]. Thiazolidinediones, especially rosiglitazone but also possibly pioglitazone[7] are linked to an increased incidence of fractures.

Canagliflozin's label has been updated by the US Food and Drug Administration to incorporate information about fracture risk and decreased bone mineral density (BMD)[8]. Given that bone health and related fracture risk have resulted in significant economic and societal costs, the impact of SGLT2 inhibitors on fractures must be thoroughly assessed.

The effects of SGLT2 inhibitors on bone metabolism, as well as their probable mechanisms, are summarised and their relationship with fracture risk is discussed. Geraniin has been proven in recent studies to help with bone growth, resorption and microstructure changes[9]. The goal of this study was to look at the bone-protective effects of geraniin as a co-treatment in diabetic rats treated with canagliflozin.

MATERIAL AND METHODS

Animals

The study used healthy male wistar albino rats that were 3- to 4-months-old and weighed 180 to 240g. The animals were obtained from King Khalid University's Central Animal House in Abha, Saudi Arabia. During the trial, the animals were kept in cages and fed a standard pellet diet and filtered water ad libitum under standard settings (light/dark cycle of 12 h/12 h with 50-70 percent humidity, at 25ºC 3ºC). For 14 days, the animals were acclimatised to the laboratory setting. The treatment was carried out in compliance with King Khalid University's animal ethics committee's approval and the US National Institute of Health's guidelines for the care and use of laboratory animals (NIH Publication No. 85-23, revised 1996).

Induction of diabetes

The pancreatic-cell toxin streptozotocin (STZ) (Sigma Chemical Co., freshly dissolved in sterile saline, 0.9 percent was injected intraperitoneally at a dose of 65mg/kg body weight to induce diabetes in the animals[10,11]. The rats in the control group were all given the same amount of vehicle. To avoid degradation, STZ was weighed separately for each animal, solubilized with 0.1ml of freshly made cold Na-citrate buffered (NaB-0.1M, pH 4.5) and delivered within 5 minutes. The STZ injection volume was calculated to be 1.0ml/kg.

To counteract the significant acute hypoglycemia effect of STZ, rats were given a 5 percent glucose solution for 48 hours following the injection. Blood was drawn from the tail vein three days after STZ injection and samples were tested for blood glucose using a glucometer (Aqua-Check, Roche).

Diabetic animals were defined as those with fasting blood glucose levels (BGLs) more than 250mg/dL. The rats were divided into three groups of six animals each: Group 1 (non-diabetic control), Group 2 (diabetic control), Group 3 (geraniin 40mg/kg body weight), Group 4 (canagliflozin 40mg/kg) and Group 5 (canagliflozin 40mg/kg + Geraniin 40mg/kg body weight). Blood glucose levels were measured once a week for the course of the trial using a Roche Accu-Chek advantage glucometer to determine the animals' hyperglycemic status. The animals that did not develop blood glucose levels greater than 250mg/dL were not included in the study. The rats in the control group (n=6) who were given saline instead of streptozotocin had normal blood glucose levels (120mg/dl).

Determination of fasting blood glucose

The rats were fasted for 12-14 hours before blood samples were taken from their tail veins to assess blood glucose levels using a glucometer. Blood will be obtained with a 1-ml needle, put on a glucose strip and quantified using a glucometer after the rats' tails have been cleansed with 70% (v/v) ethanol.

Determination of intra-peritoneal glucose tolerance test

As a baseline, all of the rats were fasted for 12-14 hours and blood was drawn from the tail vein. The rats were then intra-peritoneally administered 2g/kg body weight (BW) of a 40% (w/v) glucose solution. At 30, 60, 90 and 120 minutes following glucose

therapy, blood will be drawn from the tail vein and tested for blood glucose using a glucometer.

Diabetes was proven in these rats by fasting blood sugar levels of less than 250mg/dl.

Determination of hemoglobin A1c

Hemoglobin A1C (HbA1c) will be measured using a Clover A1cTM Self-Analyzer after blood samples from the tail vein are taken and put on a test cartridge. The percentage of HbA1c in the blood sample will be displayed on the Clover A1cTM Self-Analyzer's LCD screen.

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 glucose profiles of the positive control group (STZ) deteriorated over time (Table No.1). However, canagliflozin and geraniin, both alone and in combination, were demonstrated to protect against diabetes progression.

HBA1C levels were higher in the positive control group than in the normal control group ($p < 0.05$), as indicated in Table No.2. In contrast to the positive control group, canagliflozin and geraniin alone and in combination were shown to lower HBA1C levels, implying that geraniin plays a favourable effect.

Bone mineral density analysis results showed that the lumbar (L1-L4) and femoral bone mineral density (BMD) were decreased in diabetic rats ($p < 0.05$), which were recovered by canagliflozin and geraniin alone and in combination treatment. There was significant difference in BMD between positive group and other treatment groups (Table No.3). These results suggest that geraniin can protect the bone damage caused by antidiabetic drugs.

Statistical analysis

The results shall be expressed as mean ± standard deviation (SD). Data obtained from various groups shall be statistically analysed using one way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. The '*p*' value of less than 0.05 shall be considered statistically significant.

Discussion

Canagliflozin was linked to an increased risk of fractures in diabetic individuals, especially in the upper and lower extremities, which was driven by a significantly greater fracture incidence in individuals with a high risk of cardiovascular disease.

Although the cause of the increased fracture risk with canagliflozin is unknown, the small, inconsistent changes in total hip BMD (but not femoral neck, lumbar spine, or distal forearm BMD) seen with canagliflozin over 104 weeks, as well as the fact that an early increase in fractures was seen in only a subgroup of patients treated with canagliflozin, suggest that extrinsic factors related to canagliflozin[12]. For the first time, evidence of geraniin's preventive action against canagliflozin-induced bone loss is presented in this study. The preventive impact of geraniin on canagliflozin-induced bone loss in diabetic male rats was investigated in this study. The findings suggest that geraniin, in conjunction with canagliflozin, can increase BMD and bone quality while also controlling blood glucose.

In rats, geraniin was found to exhibit bone-protective properties[9]. However, no studies have been done to see if geraniin can protect against diabetes-induced osteoporosis. Our findings showed that an 8-week geraniin therapy can reduce bone loss in diabetic rats.

In previous research, we discovered reduced BMD in diabetic rats when compared to normal rats. BMD was lowered by canagliflozin, especially in the femur and lumbar vertebrae. After therapy with geraniin, the negative effects of canagliflozin on femur-BMD were completely reversed. However, it does show that these bone-protecting actions could be due to a variety of processes. In addition, *in vivo* investigations and clinical trials should be done to learn more about the many features of this combination medicine and how it works.

Table No.1: Effect of Geraniin in combination with canagliflozin 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*	320.35 ±9.2*	320.35 ±9.2*	330.35 ±9.7*
4	Canagliflozin	200mg/kg	233.32 ±7.3*	215.24 ±9.4*	208.26 ±7.8*	192.23 ±8.2*	173.35 ±8.8*	140.31 ±9.8*	95.36 ±9.2*	95.36 ±9.2*	80.35 ±9.7*
5	Canagliflozin +Geraniin	200mg/kg, +40mg/kg	238.33 ±7.7*	225.25 ±9.6*	207.22 ±7.8*	176.28 ±8.7*	155.35 ±8.4*	130.35 ±9.6*	92.35 ±9.4*	92.35 ±9.4*	84.35 ±9.8*

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 canagliflozin 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	Canagliflozin	5.44±0.14*
5	Canagliflozin+Geraniin	5.47±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 Canagliflozin 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	Canagliflozin	70 ± 2.2*	92 ± 2.3*
5	Canagliflozin+Geraniin	138 ± 2.2*	170 ± 2.5*

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

CONCLUSION

In a diabetes-induced rat model, geraniin enhanced bone mass, while co-supplementing geraniin with canagliflozin prevented canagliflozin-induced bone loss. As a result, it is expected that co-administration of geraniin with canagliflozin as a therapeutic strategy will reduce bone loss and fracture risk in T2DM patients using canagliflozin.

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CONFLICTS OF INTEREST

"The authors state that they have no competing interests. The funders had no involvement in the study's design, data collection, analysis, or interpretation, manuscript preparation, or the decision to publish the findings".

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