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GERANIIN PREVENTS DAPAGLIFLOZIN INDUCED BONE LOSS IN DIABETIC RATS BY REDUCING BLOOD GLUCOSE AND SUPPRESSING BONE TURNOVER

Krishnaraju Venkatesan[*1], Premalatha Paulsamy[2], Rama Ramaiah[2], Kalpana Krishnaraju[3], Swetha Devidi[4]

[1*]Department of Pharmacology, College of Pharmacy, King Khalid University, Abha, Asir Province, Saudi Arabia.
[2]College of Nursing for Girls, King Khalid University, Khamis Mushayit, Asir Province, Saudi Arabia.
[3]Department of Pharmacy, Erode College of Pharmacy, Veppampalayam, Erode, India.
[4]Department of Pharmacy, St.Mary's Group of Institutions, Hyderabad, Telangana, India.

ABSTRACT

Sodium-dependent glucose transporters 2 inhibitor (SGLT2I) has been shown in recent research to have potential deleterious effects on the skeleton, including an increased risk of fracture in patients with type 2 diabetic mellitus (T2DM). Effects generated by all SGLT2I class medications, including dapagliflozin, may play a role in this risk. The purpose of this trial was to see if geraniin could help prevent dapagliflozin induced bone loss. Streptozotocin was used to induce diabetes. Diabetic rats were administered either dapagliflozin (1.0mg/kg/day) or geraniin (40mg/kg) alone or in combination for eight weeks. BMD (Bone mineral density) of the femur and lumbar vertebrae was assessed by dual-energy X-ray absorptiometry (DXA) at the end of the trial. Serum glucose and glycosylated haemoglobin serum were also tested. Both alone and in combination, the treatment significantly reduced elevated blood glucose levels. Dapagliflozin therapy significantly lowered HBA1C levels when compared to the positive control. Geraniin and dapagliflozin together significantly lowered blood glucose and HBA1C levels. In the femur and lumbar vertebrae, dapagliflozin showed unfavourable effects on BMD, but geraniin treatment considerably reduced these effects. This study suggests that geraniin supplementation could be an effective way to reduce dapagliflozin-induced bone loss in diabetics using the drug.

KEYWORDS

Dapagliflozin, T2DM patients and Bone turnover.

Author for Correspondence:
Krishnaraju Venkatesan,
Department of Pharmacology, King Khalid University, Abha, Asir Province, Saudi Arabia.
Email: kvenkatesan@kku.edu.sa

INTRODUCTION

Diabetes mellitus is a term used to describe a collection of metabolic illnesses characterised by high blood glucose levels. Among recent decades, the global prevalence of diabetes in adults has risen considerably. According to the International

Diabetes Federation's Diabetes Atlas, global estimates of diabetes prevalence and health expenditure will reach 10.4% and $642 million USD, respectively, by 2040[1].

Long-term hyperglycemia and poor glycemic control both contribute to the development of diabetic complications, such as diabetic related osteoporosis, which is characterised by a number of negative effects on bone metabolism and has serious consequences for diabetic patients in terms of decreased bone mineral density and increased fracture risk[2]. Diabetes-related osteoporosis can increase the chance of fractures, which can have a negative influence on diabetes patients' quality of life and lifespan. The notion that glucose-lowering medicines could influence bone metabolism and increase the risk of fracture in diabetic individuals was not even addressed until a few years ago.

Although anti-hyperglycemic medicines are given for their effect on blood glucose, it has been revealed that some anti-hyperglycemic medicines have the ability to alter bone metabolism either positively (Metformin or Irbesartan) or negatively (Thiazolidinediones)[3,4]. As a result, research into the risks and benefits of anti-diabetic medications is required. On January 8, 2014, the FDA (Food and Drug Administration) approved dapagliflozin as an SGLT2I (sodium-dependent glucose co-transporter type 2 inhibitor) to treat T2DM. This class of medicines lowers blood glucose levels in diabetic patients by reducing filtered glucose reabsorption in the renal proximal tubule, irrespective of insulin action or secretion, and with a lower risk of hypoglycaemia[5].

Dapagliflozin has gotten a lot of attention from researchers as a medication class with a unique mechanism for treating hyperglycemia. But, dapagliflozin has been shown to increase the risk of fractures in T2DM individuals with mild renal impairment in a study[6]. According to an FDA document, SGLT2I treatment can raise serum levels of PTH and FGF23, causing additional bone deterioration in diabetes individuals[7]. Recent research has shown that geraniin can help with bone development, resorption, and microstructure alterations[8]. The goal of this study was to determine how dapagliflozin influenced BMD in dapagliflozin-treated rats and how geraniin affected BMD in dapagliflozin-treated rats.

MATERIAL AND METHODS[9-10]

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

To counteract the drug's significant acute hypoglycemia effect, rats were given a 5% glucose solution for 48 hours after receiving STZ. The study did not include the animals that 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 (around 120mg/dl).

Determination of fasting blood glucose

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 were drawn using a 1-ml needle 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, dapagliflozin have been shown to protect against diabetes development.

HBA1C levels were higher in the positive control group than in the normal control group (p 0.05), as indicated in Table No.3. In contrast to the positive control group, dapagliflozin and geraniin, alone and in combination, were shown to lower HBA1C levels, implying that geraniin plays a favourable 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 dapagliflozin 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

Diabetes-related osteoporosis, which increases the risk of fractures, is a prevalent comorbidity in individuals with T1DM or T2DM, and is likely the result of a number of factors, including persistent hyperglycemia[11]. According to Moayeri's research, there is a link between T2DM and overall fractures in patients and these findings highlight the necessity for fracture prevention techniques in diabetic patients[12].

The potential profits and hazards of SGLT2I as a novel anti-diabetic medication class are not yet completely understood. A variety of clinical trials using several SGLT2I medications were conducted, with mixed outcomes. Diabetes is no longer a problem. Canagliflozin, dapagliflozin, and empagliflozin are the only three SGLT2I medicines currently licenced for the treatment of T2DM. In patients treated with these SGLT2Is, a retrospective pooled dataset analysis of placebo-controlled Phase III studies treated adult T2DM patients with either canagliflozin or dapagliflozin found modest, but clinically insignificant increases in serum phosphate, magnesium, and osteocalcin concentrations[13,14].

Geraniin was discovered to have bone-protective effects in rats[8]. However, no research has been done

to examine if geraniin can protect against osteoporosis caused by diabetes. In previous studies, dapagliflozin reduced BMD, particularly in the femur and lumbar vertebrae. The deleterious effects of dapagliflozin on femur-BMD were entirely reversed after treatment with geraniin.

SGLT2I has attracted attention as a novel class of anti-diabetic medicine with varied therapeutic potential, but the danger has remained unknown due to a lack of animal and clinical data. Under the T2DM scenario, there are still certain disparities in physiology and metabolic profiles between rodents and humans (T2DM patients often suffered from hyperuricacidemia while rodent do not).More clinical trials and animal studies are needed before a conclusion can be formed on the differences in efficacy and safety between SGLT2Is. For the first time, this study provides evidence for the preventative impact of geraniin against dapagliflozin-related bone loss. In addition, *in vivo* studies and clinical trials are recommended to uncover the complex aspects of this combination therapy and its mechanism.

Table No.1: Effect of Geraniin in combination with Dapagliflozin 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	Dapagliflozin	200mg/kg	243.32 ±7.3	235.23 ±8.2*	215.22 ±5.8*	210.24 ±8.9*	180.32 ±7.8*	150.35 ±8.8*	126.32 ±10.6*	101.33 ±8.2*	90.35 ±8.7*
5	Dapagliflozin + Geraniin	200mg/kg, +40mg/kg	218.33 ±7.7*	208.24 ±8.3*	159.22 ±7.5*	160.26 ±8.6*	125.35 ±8.3*	100.39 ±9.4*	89.33 ±10.2*	82.35 ±9.5*	85.35 ±9.5*

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 Dapagliflozin 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	Dapagliflozin	5.45±0.14*
5	Dapagliflozin +Geraniin	5.42±0.17*

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 Dapagliflozin 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	Dapagliflozin	80 ± 2.2*	100 ± 2.4*
5	Dapagliflozin + Geraniin	130 ± 2.7*	170 ± 2.1*

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

CONCLUSION

Geraniin increased bone mass in a diabetes-induced rat model, and co-supplementing geraniin with dapagliflozin reduced dapagliflozin-induced bone loss. As a result, co-administration of geraniin with dapagliflozin as a therapeutic strategy is likely to minimise bone loss and fracture risk in T2DM patients who are on dapagliflozin.

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CONFLICT 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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