



QUERCETIN IMPROVES POSTPRANDIAL HYPERGLYCEMIA IN RATS TREATED WITH HIGH-DOSE GLUCOCORTICOID

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Summary

Background: Glucocorticoid-induced hyperglycemia is a common clinical problem among glucocorticoid users. The present study was designed to explore whether oral administration of quercetin, a major member of flavonoid family, could correct hyperglycemia in rats treated with high-dose methylprednisolone.

Methods: thirty two Sprague-Dawley rats were randomized into four groups of eight each and treated for six weeks with one of the followings: 1) normal saline as placebo; 2) 40 mg/kg methylprednisolone sodium succinate (MP); 3) MP + 50 mg/kg quercetin and 4) MP + 150 mg/kg quercetin. Methylprednisolone was injected subcutaneously and quercetin was orally gavaged. All treatments were given three days a week.

Results: At the end of the study, 150 mg/kg quercetin significantly decreased postprandial plasma glucose (84.9 ± 11.8 vs. 102.8 ± 11.5 , $p = 0.02$) and as well elevated insulin concentration compared with MP group (5.2 ± 1.4 vs. 3.5 ± 0.6 ng/ml; $p = 0.02$). Administration of 50 mg/kg quercetin did not show such effects.

Conclusion: These data suggest that quercetin intake with a dose of 150 mg/kg can be considered as a protective agent for glucocorticoid-induced hyperglycemia mostly due to its stimulating effect on pancreatic β -cells to excrete more insulin hormone.

Key words: Quercetin; Hyperglycemia; Glucocorticoid; Rat

Introduction

Glucocorticoid-induced hyperglycemia (GIH) is a common clinical problem among glucocorticoid users. The odds ratio for new-onset diabetes mellitus in glucocorticoid-receiving patients ranges from 1.5 to 2.5 and the risk increased proportionally with increasing glucocorticoid dosage (1). Nowadays, glucocorticoids (GCs) such as prednisone, methylprednisolone, and dexamethasone are widely used as a treatment for numerous clinical disorders, including pulmonary, gastrointestinal, rheumatologic and renal diseases mostly due to their anti-inflammatory and immunomodulatory properties. Although, these drugs have many benefits to stop inflammation, their damaging adverse effects such as hyperglycemia/diabetes, hypertension, hyperlipidemia, osteoporosis, muscle wasting and obesity must be taken seriously (2). Hyperglycemic effect of glucocorticoid drugs might be due to several mechanisms such as: increasing hepatic glucose production (gluconeogenesis) (3), decreasing glucose uptake into muscle and adipose tissue (4), inhibiting insulin secretion, inducing apoptotic death of pancreatic β -cells (5, 6), changing and downregulating of insulin receptors (7). Mild hyperglycemia may not require treatment if the steroids will be discontinued in a few weeks. However, oral agents or insulin must be prescribed for moderate and severe hyperglycemia in patients undergoing glucocorticoid therapy for a long period of time (8).

Previous studies suggest that some plant derived compounds can improve postprandial glucose levels in experimental type 1 and type 2 diabetes mellitus. Some of these natural extracts have protective effects against pancreas cells damages (9). Some others such as grape, pine, chestnut and curcumin are known to be α -glucosidase inhibitors, which can retard the liberation of glucose from dietary carbohydrates and delay glucose absorption, resulting in reduced postprandial plasma glucose levels (10-12). Flavonoids as the most important bioactive plant pigments were also studied for their α -glucosidase inhibitory activity

and it was reported that rat's intestinal α -glucosidase was inhibited by many bioflavonoids such as anthocyanidins, isoflavones, and flavones (13). Quercetin, 3,3',4',5,7-Pentahydroxyflavone; 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one, $C_{15}H_{10}O_7$, is a major member of flavonoid family isolated from onions, apples, grapes and tea. This flavonol is mostly well-known for its antioxidant and anti-inflammatory properties. Some studies indicate that quercetin can as well be beneficial for preventing hyperglycemia in experimental model of diabetes mellitus. Oral administration of 100 mg/kg quercetin could attenuate fasting and postprandial glucose levels in experimental diabetes mellitus, at least in part by inhibiting α -glucosidase activity (14). In addition, extract of onion skin containing 6.04 g quercetin/100 g dried weight, significantly reduced the blood glucose spike after sucrose loading (15). However, no study has evaluated so far the effect of quercetin on glucocorticoid-induced hyperglycemia. The present study was designed to explore whether oral administration of quercetin could correct glucocorticoid-induced hyperglycemia in rats treated with high-dose methylprednisolone.

Methods

Animals and chemicals

A total of 32 female Sprague-Dawley rats aged 6-7 months with an average weight of 210 ± 30 grams were purchased from Razi Institute (Karaj, Iran). The animals were housed under standard laboratory conditions with temperature $20-25^\circ\text{C}$, and a 12-h light/dark cycle (lights on 08:00). All rats had free access to tap water and pelleted commercial chow diet. The animals were cared for in accordance with the Guide to the Care and Use of Experimental Animals. The experimental protocol was approved by The Ethical Committee of the Tehran University of Medical Sciences. Efforts were made to use the minimum number of animals and to minimize their pain and suffering.

Quercetin, with a purity of 95%, was obtained

from Sigma-Aldrich Chemicals (St. Louis, MO, U.S.A). The quercetin suspension was prepared by adding quercetin in 0.05% aqueous carboxy methyl cellulose (CMC) solution immediately before being orally gavaged. Glucocorticoid was used in the form of methylprednisolone sodium succinate, SOLU-MEDROL, obtained from Pfizer Pharmaceuticals (NY, U.S.A).

Study design

Thirty two animals were randomized into four groups each containing eight rats and treated for six weeks. All groups were injected subcutaneously (s.c.) with methylprednisolone sodium succinate (40 mg/kg body weight) except the control group which received normal saline. Each of the three glucocorticoid-injected groups followed one of these treatments: 1- CMC (per os: p.o.) as quercetin placebo, 2- quercetin (50 mg/kg, p.o.), 3- quercetin (150 mg/kg, p.o.). All treatments were given three days a week. At the end of the study animals were anesthetized with intraperitoneal (i.p.) injection of ketamine (50 mg/kg) together with xylazine (30 mg/kg). Blood samples were collected by cardiac puncture and were immediately centrifuged for serum isolation.

Biochemical analysis

Serum samples were stored at -80°C until they were analyzed for postprandial glucose level (Glucose kit, Ziestchem Diagnostics co. Tehran, Iran). Commercially available ELISA kits were used to measure insulin concentration (Demeditec Diagnostics GmbH, Germany).

Statistical analysis

All data were presented as mean \pm standard deviation (SD). The Statistical Package for Social Sciences (version 18.0; SPSS Inc., Chicago, USA) was used for all analyses. Statistical differences between groups were assessed using analysis of variance (ANOVA) followed by Bonferroni post hoc test. A Pearson correlation was used to explore the association between postprandial glucose with insulin level. All assumptions such as normality and equality of variances were fulfilled. Statistical significance

was set at $p < 0.05$.

Results

Following six weeks of methylprednisolone injection, the mean postprandial plasma glucose level was magnified drastically in glucocorticoid-treated animals (102.8 ± 11.5 mg/dl) compared with the control group (73.4 ± 10.3 mg/dl; $p < 0.001$). High dose of quercetin (150 mg/kg) could improve hyperglycemia in comparison with MP group (84.9 ± 11.8 vs. 102.8 ± 11.5 mg/dl, $p = 0.02$). In addition, at the end of the experiment, a modest non-significant decline was observed in the serum insulin level of MP-treated rats comparing controls (3.5 ± 0.6 vs. 4.0 ± 1.0 $\mu\text{IU/dl}$). Nevertheless, insulin level significantly ascended in Q150 group (5.2 ± 1.4 $\mu\text{IU/dl}$). A lower dose of 50 mg/kg did not show any effect on plasma glucose or insulin levels (Table 1). The postprandial glucose was negatively related by Pearson correlation to the changes in insulin level ($r = -0.40$, $p = 0.02$). In other words, insufficient amount of insulin in GC-treated rats resulted in higher glucose levels.

Table 1 – Postprandial plasma glucose and insulin levels at the end of the experiment

	Control	MP	MP+Q50	MP+Q150	p value
PPG	73.4 \pm 10.3 ^b	102.8 \pm 11.5 ^a	109.6 \pm 12.1 ^a	84.9 \pm 11.8 ^b	< 0.001
Insulin	4.0 \pm 1.0	3.5 \pm 0.6	4.2 \pm 0.8	5.2 \pm 1.4 ^b	0.017

Data are presented as Mean \pm SD. n=8 for all groups. PPG, postprandial plasma glucose; MP, methylprednisolone; Q50, quercetin 50 mg/kg; Q150, quercetin 150 mg/kg; Analysis of variance (ANOVA) followed by Bonferroni test.

^a $p < 0.05$ compared with control group

^b $p < 0.05$ compared with MP group

Discussion

According to our data, methylprednisolone injection for six weeks caused a considerable augmentation in postprandial glucose and an inconspicuous plunge in insulin level. Glucocorticoids, as widely prescribed anti-inflammatory drugs, are the most common cause of

drug-induced diabetes and hyperglycemia. Hyperglycemic effect of GCs might be due to several mechanisms such as: increasing hepatic glucose production (gluconeogenesis) (3), decreasing glucose uptake into muscle and adipose tissue (4), inhibiting insulin secretion, inducing apoptotic death of pancreatic β -cells (5, 6), changing and down-regulating of insulin receptors(7). Normally, GCs administration results in higher insulin production in healthy people because they act as counter-regulatory hormones and increase the body's demand for insulin (16). But, in high doses, they have been shown to decrease insulin secretion as a result of the compensatory failure of pancreatic β -cells (17). In addition, high levels of circulating glucose and free fatty acids, which are common implication in GC users, are toxic to β -cells and cause not only their dysfunction but also their decreased number due to induction of apoptosis (18). Our findings are in agreement with this body of evidence.

In this study, we examined the ability of two different doses of quercetin, as a bioactive plant-derived component, to prevent GC-induced hyperglycemia in rats. After six weeks of experiment, 50 mg/kg quercetin did not have any significant effect on postprandial plasma glucose or insulin levels. However, the higher dose of 150 mg/kg could normalize postprandial plasma glucose (PPG) and noticeably increase insulin level compared with GC-treated group. It was previously reported that dietary polyphenols including flavonoids, phenolic acids, anthocyanidins and so on, may influence carbohydrate metabolism at many levels. Some of these phytochemicals, which can be found abundantly in plant-based foods, can influence digestion and absorption of carbohydrates.

They can retard the release of glucose by inhibiting α -amylase and α -glucosidase as the key enzymes responsible for dietary carbohydrates digestion, resulting in reduced postprandial plasma glucose. Onions, grapes, pine, chestnut, berries and tea were reported to decrease PPG due to their polyphenolic components (10-12, 19). Quercetin was also known as α -amylase and α -glucosidase inhibi-

tor(14). Therefore, its improving impact on PPG that was observed in the present study might be at least partially associated with its ability to impair carbohydrates absorption.

Polyphenols might also have the potential to preserve β -cells. Since the loss and dysfunction of β -cells play a pivotal role in GC-induced hyperglycemia, protection and induction of β -cell proliferation is one of the most important strategies to prevent diabetes. Genistein, a flavonoid widely found in legumes such as soy bean, has been previously reported to ameliorate diabetes and improve glucose tolerance. It caused about 2-fold increase in circulating insulin levels, which could be due to improved insulin secretion, greater islet β -cell proliferation and reduced β -cell apoptosis (20). Quercetin was also shown to increase insulin secretion and preserve pancreatic β -cell integrity in experimental diabetes (21). Furthermore, quercetin, as a strong antioxidant and anti-inflammatory agent, can protect pancreatic cells by suppressing oxidative stress and down-regulating inflammatory gene expression (22).

Taken together, the present study revealed for the first time that oral administration of 150 mg/kg quercetin can attenuate glucocorticoid-induced hyperglycemia and normalize postprandial plasma glucose. This phenomenon seems to be partially due to the protective effect of quercetin against pancreatic β -cells loss and dysfunction and also stimulating them for more insulin secretion.

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