Antidiabetic effects of quercetin in streptozocin-induced diabetic rats

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Received 7 February 2003; received in revised form 12 June 2003; accepted 18 June 2003

Abstract

Effects of the intraperitoneal injection of quercetin in streptozocin-induced diabetic and normal rats were investigated and compared. Although quercetin had no effect on plasma glucose level of normal animals, it significantly and dose-dependently decreased the plasma glucose level of streptozocin-induced diabetic rats. Glucose tolerance tests of the diabetic animals approached those of normal rats, their plasma cholesterol and triglycerides were reduced significantly, while their hepatic glucokinase activity was significantly increased upon quercetin treatment. In normal rats, quercetin did not affect the glucose tolerance test, but resulted in an increase of plasma cholesterol and triglycerides and a decrease in hepatic glucokinase activity. No significant pathologic changes were noted in hepatocytes or kidney tubules and glomeruli, while the number of pancreatic islets significantly increased in both treated normal and diabetic groups. It is concluded that quercetin, a flavonoid with antioxidant properties brings about the regeneration of the pancreatic islets and probably increases insulin release in streptozocin-induced diabetic rats; thus exerting its beneficial antidiabetic effects. However, it may be of little value in normoglycemic animals.

Keywords: Cholesterol; Diabetes; Glucose tolerance; Hepatic glucokinase; Pancreatic islets; Quercetin; Streptozocin; Triglycerides

1. Introduction

The most common substances inducing diabetes in the rat are alloxan and streptozocin (streptozotocin, STZ). STZ is taken up by pancreatic β-cells via glucose transporter GLUT2. The main cause of STZ-induced β-cell death is alkylation of DNA by the nitrosourea moiety of this compound. However, production of NO and reactive oxygen species may also be involved in DNA fragmentation and other deleterious effects of STZ. The toxic action of alloxan on pancreatic β-cells involves several processes such as oxidation of essential –SH groups, inhibition of β-cell glucokinase, generation of free radicals and disturbances in intracellular calcium homeostasis. Calcium does not play a significant role in necrosis of β-cells by STZ since calcium channel antagonists do not protect β-cells against this drug (Szkudelski, 2001). Interestingly, STZ is not equally effective in all vertebrates and mechanisms of action may differ between groups (Wright et al., 1999).

β-Cells are affected by many immunological and chemical agents leading to local inflammations producing IL-6 and glucocorticoids. IL-6/glucocorticoid stimulation produces an active transcriptional complex for Reg, a β-cell regenerating factor gene, in which poly (ADP-ribose) synthetase/polymerase (PARP) is involved. In the presence of PARP inhibitors such as nicotinamide when PARP is not itself poly (ADP-ribose)-ated,
the transcriptional complex is stabilized and Reg gene transcription and subsequent Reg protein formation occurs in β-cells. This protein acts as a growth factor on β-cells via Reg receptor. DNA replication in β-cells takes place and β-cell regeneration is accomplished. DNA damaging substances such as superoxide (O2·−) and nitric oxide (NO·) are produced in inflammatory processes by cytotoxic agents such as STZ. When DNA is damaged, PARP senses the nicks and autopoly (ADP-ribosyl)-ates itself for DNA repair. Autopoly (ADP-ribosylation) of PARP inhibits the formation of Reg gene transcriptional complex and transcription of this gene stops (Akiyama et al., 2001).

Chemicals with antioxidant properties and free radical scavengers in particular protect autopsy (ADP-ribosyl)-ation of PARP and by stabilizing Reg gene transcriptional complex, result in the regeneration of β-cells and protect pancreatic islets against cytotoxic effects of STZ or alloxan (Szkudelski, 2001).

Recently, there has been renewed interest in the use of plant compounds as antidiabetic compounds (Pari and Saravanan, 2002). Flavonoids are naturally occurring phenolic compounds that are widely distributed in plants. Due to the presence of aromatic hydroxyl groups, flavonoids have strong antioxidant properties. They are scavengers of reactive oxygen and nitrogen species and, therefore, inhibit peroxidation reactions. They also protect macrophages from oxidative stress by keeping glutathione in its reduced form (du Thie and Crozier, 2000; Fuhrman and Aviram, 2001).

Flavonoids have the capacity to inhibit enzymes such as cyclooxygenases and protein kinases involved in cell proliferation and apoptosis (Formica and Regelson, 1995). It was reported that a flavonoid, (−)-epicatechin, protects normal rat islets from alloxan, normalizes blood glucose levels and promotes β-cell regeneration in islets of alloxan—treated rats (Chakravarthy et al., 1981, 1982a,b). Tritiated thymidine incorporation into islet cell DNA was also enhanced by this flavonoid in an in vitro study (Hii and Howell, 1984). However, the beneficial effects of (−)-epicatechin in STZ—diabetic animals could not be demonstrated (Bone et al., 1985). A different flavonoid, quercetin, used in doses of 10–50 mg/kg body mass was capable of normalizing blood glucose level, augmenting liver glycogen content and significantly reducing serum cholesterol and LDL concentration in alloxan–diabetic rats (Nuraliev and Avezov, 1992).

Hii and Howell (1985) showed that exposure of isolated rat islets to certain flavonoids such as (−)-epicatechin or quercetin enhances insulin release by 44–70%. They argue that such flavonoids may act on islet function, at least in part, via alteration in Ca2+ fluxes and in cyclic nucleotide metabolism.

Due to differences in the mechanism of action of streptozocin and alloxan and the presence of controversial reports on the antidiabetic effects of different flavonoids in alloxan—treated and STZ—induced diabetes, we decided to re-evaluate the effects of two different doses of quercetin in STZ—induced diabetic rats and compare the results with the effects of this flavonoid on normoglycemic animals.

This study compares the action of quercetin on blood glucose concentration, glucose tolerance test, pancreatic islet regeneration, activity of an insulin-induced enzyme (hepatic glucokinase), and the levels of plasma cholesterol and triglycerides (TG) in normoglycemic and diabetic rats.

2. Materials and methods

2.1. Reagents

Glucose and fatty acid free bovine serum albumin were purchased from Roche chemical company (Germany). Leuconostoc mesenteroides glucose 6-phosphate dehydrogenase (G6PD), Na2,ATP, quercetin, HEPES buffer and dithiothreitol were obtained from Sigma Chemical Company (St. Louis, MO, USA). Na2,NAD was from Fluka chemical company (Switzerland), and streptozocin vials containing 1 g streptozocin and 220 mg citric acid was obtained from Upjohn Co. (Kalamazoo, MI, USA). Streptozocin was reconstituted with 9.5 ml of 0.9% NaCl solution to pH 3.5–4.5 according to manufacturer’s instructions. Furthermore, dilution of the drug was performed in 0.9% NaCl solution immediately before use. The enzymatic kits for the determination of glucose, cholesterol and triacylglycerol (TG) were purchased from Pars Azemoon Co. (Tehran, Iran).

2.2. Animal experiments

Adult male Sprague–Dawley derived rats bred and raised at the University animal quarters with
a mass ranging from 200 to 220 g were used. Five animals each were housed in a cage and fed a rat chow diet (Pars Dam Co, Tehran, Iran) and given water ad libitum. Diabetes was induced upon injection of streptozocin (40 mg/kg body mass) through the caudal vein two weeks prior to the initiation of treatments. Blood was obtained from the tail vein using heparinized microhematocrit tubes. Plasma glucose of all groups was measured weekly. After 2 weeks, animals with plasma glucose level exceeding 16.6 mM were considered as diabetic. Normoglycemic and streptozocin diabetic rats were employed for the tests described below.

2.3. Effect of quercetin on plasma glucose level

The required amount of quercetin was dissolved in 0.5 ml of 60% ethanol prior to injection to rats. Five groups of rats including two normoglycemic and three diabetic groups (five rats per group) were used: (1) normoglycemic control group receiving the vehicle as one intraperitoneal (i.p.) injection of 0.5 ml 60% ethanol per day; (2) normoglycemic rats receiving one i.p. injection of 10 mg quercetin per kg body mass per day; (3) diabetic control rats receiving one i.p. injection of 0.5 ml 60% ethanol per day; (4) diabetic experimental animals receiving one i.p. injection of 10 mg quercetin per kg body mass per day; (5) diabetic experimental animals receiving one i.p. injection of 15 mg quercetin per kg body mass per day. Such treatments were continued for 10 days at 13:00 h every day. Blood was collected from the tails using heparinized microhematocrit tubes and used for plasma glucose determination prior to quercetin treatment (day 0) and every other day thereafter.

2.4. Glucose tolerance test

Glucose tolerance test was performed according to the procedures of Young et al. (1995) on the five different groups mentioned above. Following treatment for 10 days, plasma glucose was determined, the animals were treated with another respective dose of quercetin and deprived of food for 24 h. After this period, blood was collected through heparinized tubes for plasma glucose (0 time glucose level), cholesterol and triglyceride determinations. The fasting plasma glucose levels of the diabetic control rats, the diabetic experimental group receiving 10 mg quercetin per kg and the diabetic animals receiving 15 mg quercetin per kg were 26.8 ± 1.2, 5.9 ± 0.17 and 5.8 ± 0.44 mM, respectively. The fasting plasma glucose levels of the normoglycemic control and the normoglycemic experimental groups were in the range of 4.4–5.0 mM. Fasted animals were fed 1 ml of a glucose solution containing 3.5-mmol glucose (3 g/kg body mass) through a gavage. Tail blood were collected at 45, 90 and 135 min after glucose feeding and plasma glucose levels were determined and used for establishing glucose tolerance curves.

2.5. Hepatic glucokinase and hexokinase assay

Five groups of five rats each consisting of two normoglycemic and three diabetic groups were injected i.p. with various levels of quercetin as described above except that injections were done into normoglycemic animals, diabetic animals receiving 10 mg quercetin per kg body mass, and diabetic animals receiving 15 mg quercetin per kg body mass were continued for 8, 10 and 7 days, respectively. Plasma glucose levels were monitored during the treatments. At the times specified above, when the plasma glucose level of the quercetin treated diabetic animals in the fed state reached approximately 7.0 mM, the animals were killed by decapitation and blood collected for serum alanine aminotransferase (ALT) assay. Livers were removed; a portion was placed in buffered 10% formalin and the rest was frozen at −70 °C and later employed for hexokinase and glucokinase assays. The pancreas and kidneys from these rats were placed in buffered 10% formalin and used for histological studies.

Frozen liver tissue (1 g) from each rat, was cut into small pieces and homogenized at 4 °C in 9 ml of a cold buffer solution (pH 7.4) containing Na-HEPES, 50 mM; KC1, 100 mM; EDTA, 1 mM; MgCl$_2$, 5 mM and dithiothreitol, 2.5 mM using a glass–Teflon Potter homogeniser with 20 up and down motions at half maximum speed. The suspension formed was centrifuged at 12 000×g for 1 h at 4 °C. The clear supernatant solution was immediately employed for the measurement of hexokinase and glucokinase activities by the coupled enzyme assay procedures of Davidson and Arion (1986). The incubation mixtures for the assay of these enzymes in both control and test tubes contained the following ingredients in a final volume of 1.0 ml: incubation buffer, pH 7.4 containing HEPES, 50 μmol; KC1, 100 μmol;
MgCl$_2$, 7.5 µmol and dithiothreitol, 2.5 µmol; fatty acid free bovine serum albumin, 10 mg; NAD$^+$, 0.5 µmol; G6PD, 4 units; liver supernatant, 100 µl for hexokinase assay or 10 µl for total hexokinase and glucokinase assays; and D-glucose, 0.5 µmol for hexokinase and 10 µmol for total enzyme activities. Tubes were preincubated at 25 °C for 5 min. To the control tubes, 0.2 ml of a solution containing 0.5 µmol of ATP was added. The increase in absorbance at 340 nm was followed for 15 min 0.5 in the test tubes, 0.2 ml of a solution containing fatty acid free bovine serum albumin, 10 mg; MgCl$_2$, 7.5 µmol and dithiothreitol, 2.5 µmol; 360 M. Vessal et al. / Comparative Biochemistry and Physiology Part C 135 (2003) 357–364

2.6. Plasma triglycerides, cholesterol and serum alanine transaminase

Plasma cholesterol and triglycerides (TG) were measured on tail blood obtained from fasted animals using enzymatic kits (Pars Azemoon Co., Tehran, Iran) prepared according to the procedures of Allain et al. (1974) and Fossati and Prencipe (1982), respectively. Serum alanine aminotransferase was measured on the blood obtained from fed animals killed for hepatic glucokinase assay using a kit (Zist Chemie, Tehran, Iran) prepared according to the procedure of Reitman and Frankel (1975) and the results were expressed in units per liter. One unit of the enzyme corresponds to the amount of the enzyme producing 1 nmol of NADH per min under assay conditions at 25 °C. Hexokinase activities were subtracted from the total hexokinase+glucokinase activities to obtain glucokinase activities. Protein concentration in the liver supernatants was measured using the biuret reagent (Gornall et al., 1949) using bovine serum albumin as a standard. Enzyme specific activities were expressed in mU per mg protein.

2.7. Histological studies

Pancreas, liver and kidneys were removed and immediately immersed in 10% formalin. After paraffin embedding, 5 µm thick sections were prepared and stained by hematoxylin and eosin. Pancreatic tissue was serially sectioned, the 5th, 10th and 15th sections of each pancreas were stained and the total number of islet cells in each section was counted under light microscope. Using graph paper, the total area of each stained section was determined and the average number of pancreatic islets per cm$^2$ was calculated.

2.8. Statistical analyses

Using SPSS version 10 software, the data were statistically analyzed by one-way ANOVA and Duncan test. The level of statistical significance was set at $P<0.05$.

3. Results

Quercetin had no effect on plasma glucose concentration of normoglycemic animals, but significantly reduced blood glucose level of diabetic rats in 8–10 days at the two doses used. The blood glucose level of the diabetic animals treated with quercetin reached normal values at the end of this period (Fig. 1).

Quercetin exerted no effect on the glucose tolerance curve of the normoglycemic animals, but at the two doses used, normalized the glucose tolerance curves of the streptozocin diabetic rats (Fig. 2). We noticed no significant difference between the effects of 10 or 15 mg quercetin/kg body mass. As seen in Fig. 2, ten days of i.p. injection of quercetin was able to normalize glucose tolerance curves of diabetic animals.

Table 1 shows the effect of quercetin on normoglycemic and STZ-diabetic rats. Quercetin treatment had no effect on hepatic hexokinase activity of normoglycemic animals, but significantly increased hexokinase activity of diabetic animals and brought the activity to the level of normoglycemic animals. Although quercetin treatment decreased the specific activity of hepatic glucokinase in normoglycemic animals, it significantly and dose-dependently increased the activity of this enzyme in diabetic animals and at 15 mg quercetin/kg b.m., glucokinase activity of the diabetic animals reached that of normoglycemic rats. Similarly, plasma cholesterol and triglyceride levels of normoglycemic animals were significantly increased by quercetin treatment, while those of diabetic animals were significantly reduced by this drug. Serum ALT levels were not changed upon the treatment of either normoglycemic or diabetic animals with this antioxidant.
Fig. 1. Plasma concentration–time profile of glucose in normoglycemic and streptozocin—diabetic rats after i.p. administration of quercetin. Each point represents the mean ± S.E.M. of five rats. ●, normoglycemic control; ○, normoglycemic + 10 mg quercetin per kg; ▲, diabetic control; △, diabetic + 10 mg quercetin per kg; ■, diabetic + 15 mg quercetin per kg.

A significant increase in the number of pancreatic islets was seen in both normoglycemic and diabetic rats treated with quercetin.

Fig. 3a and b demonstrate typical pancreatic sections of the diabetic and quercetin treated diabetic groups, respectively. There was considerably higher number of micro-islet formation in treated cases and no apparent mitotic figures were noted in these sections. Occasionally some islets were seen adjacent to the excretory ducts. The number of large and small size islets was also higher in the quercetin treated animals. Except for the presence of scattered and focal eosinophils in pancreatic septa, no specific pathologic change was noted in the quercetin treated pancreas (Fig. 3b). No significant pathological damage to hepatocytes, kidney tubules and glomeruli was observed. Minor subtle changes such as mild degree of hydropic change and higher frequency of one or two macronucleoli were noted in liver tissues treated with quercetin.

4. Discussion

The plasma glucose lowering effects of quercetin in streptozocin-induced diabetic rats and its lack of effects on plasma glucose level of normoglycemic animals (Fig. 1) is in agreement with the results of Chakravarthy et al. (1981, 1982a,b) on similar effects of (−)-epicatechin, in alloxan diabetic rats and also in accord with the data of Nuraliev and Avezov (1992) on hypoglycemic effects of quercetin in alloxan diabetic animals. However, the latter authors used 10 and 50 mg of quercetin per kg in their treatment of diabetic animals, instead of the 10 and 15 mg used in this experiment. Normalization of glucose tolerance curves in diabetic animals treated with either 10
Fig. 2. Glucose tolerance curves of normoglycemic and streptozocin—diabetic rats after i.p. administration of quercetin. Each point represents the mean ± S.E.M. of five rats. ●, normoglycemic control; ○, normoglycemic + 10 mg quercetin per kg; ▲, diabetic control; △, diabetic + 10 mg quercetin per kg; ■, diabetic + 15 mg quercetin.

Table 1
Effect of quercetin on normoglycemic and STZ-diabetic rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Hexokinase Specific activity (mU/mg protein)</th>
<th>Glucokinase Specific activity (mU/mg protein)</th>
<th>Plasma cholesterol (mmol/l)</th>
<th>Plasma TG (mmol/l)</th>
<th>Serum ALT (U/l)</th>
<th>Number of pancreatic islets (cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoglycemic control</td>
<td>0.89 ± 0.05ₐ</td>
<td>1.22 ± 0.11ₐ</td>
<td>1.5 ± 0.11ₐ</td>
<td>0.70 ± 0.02ₐ</td>
<td>13.4 ± 0.24</td>
<td>43.4 ± 3.9ₐ</td>
</tr>
<tr>
<td>Normoglycemic + 10 mg quercetin /kg b.m.</td>
<td>0.74 ± 0.07ₐ</td>
<td>0.83 ± 0.05ₐ</td>
<td>2.1 ± 0.10ₐ</td>
<td>0.79 ± 0.01ₐ</td>
<td>14.2 ± 0.12</td>
<td>61.8 ± 4.1ₐ</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>0.31 ± 0.03ₐ</td>
<td>0.23 ± 0.03ₐ</td>
<td>5.1 ± 0.08ₐ</td>
<td>1.60 ± 0.08ₐ</td>
<td>14.7 ± 0.30</td>
<td>14.8 ± 1.6ₐ</td>
</tr>
<tr>
<td>Diabetic + 10 mg quercetin/kg b.m.</td>
<td>0.75 ± 0.08ₐ</td>
<td>0.50 ± 0.04ₐ</td>
<td>2.5 ± 0.06ₐ</td>
<td>0.70 ± 0.01ₐ</td>
<td>14.9 ± 0.40</td>
<td>22.6 ± 1.2ₐ</td>
</tr>
<tr>
<td>Diabetic + 15 mg quercetin/kg b.m.</td>
<td>0.80 ± 0.02ₐ</td>
<td>1.37 ± 0.20ₐ</td>
<td>2.1 ± 0.08ₐ</td>
<td>0.60 ± 0.05ₐ</td>
<td>13.8 ± 0.50</td>
<td>33.2 ± 3.1ₐ</td>
</tr>
</tbody>
</table>

¹ For details of experimental conditions and definition of enzyme units see the text. Quercetin treatment for normoglycemic animals, diabetic rats receiving 10 mg/kg b.m. per day and diabetics receiving 15 mg/kg b.m. per day were continued for 8, 10 and 7 consecutive days, respectively.

² Data are expressed as mean ± S.E.M. of 5 rats in each group. In each column, figures bearing different superscripts are significantly different at P < 0.05 (one way ANOVA and Duncan test).
et al., 1986; Verykokidov-Vitsaropoulou and Vajias, 1986), was previously shown in our laboratory (Vessal et al., 2003).

Treatment of normoglycemic animals with 10 mg quercetin per kg lowered hepatic glucokinase activity by 32% and resulted in a significant increase in plasma cholesterol and TG (Table 1). Quercetin treatment of diabetic animals increased hexokinase activity up to 2.6-fold, glucokinase activity up to six-fold, the number of pancreatic islets up to 2.2-fold and decreased plasma cholesterol level up to 2.4-fold and TG up to 2.8-fold (Table 1). The effects of quercetin on decreasing the level of cholesterol and LDL were also demonstrated by Nuraliev and Avezov (1992). Regeneration of the islets of Langerhans by quercetin (Table 1) is in agreement with the data of Chakravarthy et al. (1981, 1982a,b) who specifically demonstrated the regeneration and the functional activity of regenerated β-cells by (−)-epicatechin. The ability of quercetin to significantly increase glucokinase activity of the liver and to reduce plasma cholesterol and TG in diabetic animals could be explained by the work of Hii and Howell (1985) on the insulin releasing capacity of quercetin in isolated rat islets of Langerhans. Increased number of pancreatic islets in quercetin treated animals may be due to increased DNA replication by the islet cells. This has been demonstrated by Hii and Howell (1984) in an in vitro study.

Quercetin resulted in an increase of islet cells in normoglycemic animals, however, it lowered glucokinase activity and increased plasma cholesterol and TG levels in those animals. Therefore, it appears that although quercetin exerts some beneficial effects on STZ-diabetic rats, it may cause some disturbances in insulin signaling of the normoglycemic animals.

It appears that quercetin as an antioxidant and a free radical scavenger prevents autopoly(ADP-ribosyl)-ation of PARP, thereby stabilizing Reg gene transcriptional complex and resulting in the regeneration of β-cells and protection of pancreatic islets against STZ or alloxan (Akiyama et al., 2001; Szkudelski, 2001).

In conclusion, it seems that quercetin, a flavonoid which produces an increase in the number of pancreatic islets, probably increases insulin release in STZ-diabetic rats and induces the hepatic glucokinase enzyme. The plasma glucose lowering property and its beneficial effects on correction of glucose tolerance test and on lowering plasma cholesterol and TG could also be probably attributed to its ability to regenerate pancreatic β-cells and to increase insulin release.

In this study, measurement of hepatic glucokinase activity was considered as an indirect index of insulin release by β-cells. However, insulin staining of the pancreatic β-cells and serum insulin estimation are required to confirm the mode of
action of quercetin in regeneration of functional β-cells and in insulin release by quercetin treated STZ-rats.

Acknowledgments

This investigation was supported by Grant No. 79–1038 from the office of Vice Chancellor for Research, Shiraz University of Medical Sciences.

References


