Quercetin ameliorates glucose and lipid metabolism and improves antioxidant status in postnatally monosodium glutamate-induced metabolic alterations

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A R T I C L E   I N F O

Article history:
Received 12 January 2012
Accepted 5 July 2012
Available online 15 July 2012

Keywords:
Monosodium glutamate
Lipid profile
Metabolism
Quercetin
Hepatoprotection
Antioxidant defenses

A B S T R A C T

We reported the effects of quercetin on metabolic and hormonal profile as well as serum antioxidant activities in a model of MSG (monosodium glutamate)-induced obesity. Rats were divided into 4 groups: MSG group, submitted to neonatal treatment with high doses of MSG, administrated subcutaneously during 10 days, from 2 day-old; control groups, which received the same volume of saline. After completing 30 day-old, these groups were subdivided into 4 groups: control and MSG groups treated and non-treated with quercetin at doses of 75 mg/kg body weight (i.p.) over 42 days. BW gain and food consumption were higher in MSG-treated rats and quercetin significantly reduced BW by 25%. While MSG increased triacylglycerol, total cholesterol and fractions, and reduced HDL concentrations, administration of quercetin normalized HDL-cholesterol and reduced others lipids. Insulin, leptin, glucose and creatinine levels were raised in MSG-treated rats and reduced after quercetin treatment. Alanine transaminase, aspartate transaminase, lactate dehydrogenase and alkaline phosphatase activities were lower after MSG-quercetin combination compared to rats given only MSG. MSG-quercetin combination augmented total protein and urea levels as well as glutathione peroxidase and superoxide dismutase activities in contrast to MSG-treated animals. Quercetin normalized serum lipid and glucose profile and minimized the MSG-related toxic effects, which was associated to its antioxidant properties.

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1. Introduction

Eating behavior plays a pivotal role in metabolic and diet-related disorders (Naim et al., 1991). Some mechanisms for food cravings may benefit mammals from nutrient deficiency, otherwise overfeeding of palatable food may cause an imbalanced intake of nutrients (Kaur and Kapoor, 2001). Monosodium glutamate (MSG) is one of the most abundant naturally occurring non-essential amino acids and MSG-treatment is able to produce metabolic changes, which can further result in severe bodily disturbances (Diniz et al., 2005).

The hypothalamus is associated with the control of food intake, energy balance and the autonomic nervous system. Among major causes of neuroendocrine obesity is the hypothalamic lesion-induced obesity (Macho et al., 2000), which can be experimentally induced after s.c. injections of MSG (Nakayama et al., 2003) or via MSG oral administration (Xu et al., 2007) to suckling rodent pups. These animal models for studying obesity often present fasting hyperinsulinemia (Maletínská et al., 2006), hyperleptinemia (Hollopeter et al., 1998), adiposity and increase of plasma fatty acids and triacylglycerols (Dawson et al., 1997). In addition, postnatal administration of MSG in rats seems to be linked to insulin resistance, body weight gain, hyperleptinemia and glucose levels (Iwase et al., 1998; Suga et al., 1999), but the exact mechanisms for these alterations are not yet clearly defined.

Although some variables, such as age, via of administration, dose and period of treatment must be taken account, it is clear that MSG is able to cause metabolic alterations. In this context some studies have shown that MSG induces oxidative stress and hepatotoxicity in rats (Onyema et al., 2006) as well as impaired...
glucose-induced insulin secretion by pancreatic islets of obese mice (Andreazzi et al., 2009). Recent findings proposed that MSG produces altered lipid profile with elevation in reactive oxygen species (ROS) formation and reduction of antioxidant activities (Park et al., 2010).

Quercetin (3,3',4',5,7-pentahydroxyflavone), a polyphenolic flavonoid compound occurring mainly in glycosidic forms (Wach et al., 2007), is a potent antioxidant found in vegetables and fruits capable of inducing hepatoprotection and also improving dyslipidemia (Amália et al., 2007). Also, it contains some phenolic hydroxyl groups that have strong antioxidant activity, functioning as a ROS scavenger itself (Boots et al., 2007). Furthermore, growing evidences has pointed to quercetin as a promoter of enhanced superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities (Amália et al., 2007). To date, no reports have focused e relationship between MSG-toxicity effects and the role of quercetin on dyslipidemia, energy balance, glucose intolerance/insulin resistance and liver metabolism.

Therefore, the present study was carried out to investigate whether quercetin is able to improve nutritional parameters, lipid and hormonal profile, liver and glucose metabolism and serum antioxidant activities of postnatally MSG-treated rats.

2. Materials and methods

2.1. Animals and experimental design

Sixty-four male Wistar rats were obtained from the Department of Biochemistry, Biosciences Institute/Campus of Botucatu, UNESP – Univ Estadual Paulista. Animals were housed in polypropylene cages with laboratory-grade pine shavings as bedding and maintained under standard conditions (12 h light/12 h dark cycle; 23 ± 1 °C room temperature). Food standard Purina (3074 SIF, Purina Ltd., Campinas, São Paulo, Brazil) and filtered water were provided ad libitum. All animals were divided into four groups (n = 16/group). Control group (CT): fed standard diet and receiving saline as vehicle; MSG group (MSG): given only MSG (4 mg/g body weight) from 2 to 12-day old; quercetin-treated group (CT + QC): receiving quercetin at doses of 75 mg/kg body weight (i.p.) started after 30-day-old, over 42 days; MSG-Quercetin group (MSG + QC): receiving MSG, from 2 to 12-day old, and quercetin as treatment, started after 30-day old, over 42 days. MSG was subcutaneously administered at the dose of 4 mg/g body weight (BW) on postnatal days 2–12 (Ebling et al., 1998). At 30 days-old, quercetin at dose of 75 mg/kg BW (Sigma, St. Louis, MO, USA) was dissolved in propylene glycol as a vehicle and injected intraperitoneally once in a week, followed by 7 days of interval over 42 days, totaling 6 applications. Food (g) and water (mL) consumption were daily measured and body weights (g) were evaluated each week during all experimental period. Experimental protocols were accepted by Ethical Committee of the Biosciences Institute /UNESP, Brazil, in accordance to the principles of the Canadian Council on Animal Care.

2.2. Biochemical and nutritional determinations

At third week quercetin-treatment were determined fasted glycemic index. After 42 days of treatment (72 days after birth), rats were fasted overnight (12–14 h) and then submitted to oral glucose tolerance test (OGTT). Glucose was orally administered by gavage (3 g/kg) at single dose as 20% aqueous solution, and then glycemia were quantified before administration and at 30, 60 and 120 min after glucose administration. Blood glucose was measured by automatic glucose analyzer (Boehringer Mannheim, Eli Lilly Ltd., São Paulo, Brazil). After OGTT, all rats were anesthetized (0.1 ml sodium pentobarbital 3%, i.p.) and euthanized by decapitation. The blood was placed into centrifuge tubes and allowed to clot to obtain the serum. Triacylglycerol (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL) were determined in serum by enzymatic method (test Kit CELM diagnosis, Modern Laboratory Equipment Company, São Paulo, SP, Brazil). The very-low-den- sity lipoprotein cholesterol (VLDL) was calculated by Friedewald equation and total protein was also quantified (Lowry et al., 1951). Low-density lipoprotein cholesterol (LDL) was selectively precipitated from 60 µL of serum by adding 1 ml of phosphotungstic acid (CELM diagnosis, Modern Laboratory Equipment Company, São Paulo, SP, Brazil) followed by centrifugation at 1400 × g for 10 min and finally read at 625 nm. Fat acids determination was achieved from acid-deproteinized samples (phosphate buffer – pH 6.7) containing chloroform. The mixture chloroform-triethylamine was filtered with sodium diethylthiocarbamate to form a colored product. Aliquots of serum was used for insulin and leptin determinations through enzyme immune assay kit (EIA kit, Cayman Chemical, USA) using an ELISA reader (Biotech Instruments, INC, USA). GSH-Px (E.C.1.1.1.9.) was assayed using 0.15 M phosphate buffer pH 7 containing 5 mM EDTA, 0.0084 M NADPH, 4 µg of

GSH-reductase, 1.125 M sodium aside and 0.15 M GSH. GSH-peroxidase unit was defined as µmol of NADPH oxidized per minute g protein. SOD (EC.1.15.1.11) activity was determined using superoxide radical (O2−) mediated nitro-blue tetrazo- lium (NBT) reduction by an aerobic mixture of NADH and phenazine methosulphate (PMS). The complete reaction system consisted of 50 mM phosphate buffer pH 7.4, 0.1 mM EDTA, 50 µM NBT, 78 µM NADH and 3.3 µM PMS. One unit of SOD was defined as the amount of protein to decrease the reference rate to 50% of maximum inhibition (Ewing and Janero, 1995). Plasma aspartate transaminase (AST – EC 2.6.1.1), alanine transaminase (ALT – EC 2.6.1.2), lactate dehydrogenase (LDH – EC 1.1.1.27), amylase (EC – 3.2.1.1), and alkaline phosphate (ALKP – EC 3.1.3.1) activities were determined using kits from Sentinelle CH (5-20155; Milan-Italy) and read at 340 nm. Finally, stored serum samples were analyzed for total protein, urea and creatinine concentrations based on kits obtained from Sentinelle CH (5-20155; Milan-Italy).

Enzyme activities were performed at 25 °C using a micro-plate reader (µQuant-MQX 200 with KJc Junior software, Bio-Tec Instruments, Winsooki, Vermont, USA). Spectrophotometric determinations were performed in a Pharmacia Biotech spectrophotometer with temperature-controlled cuvette chamber (UV/visible Ultrospec 5000 with Swift II applications, Cambridge, England, UK). All chemicals and solvents were purchased from Sigma (St. Louis, Missouri, USA).

Based on food intake and the amount of calories (Seiva et al., 2011), the following parameters were calculated:

Energy intake = mean food consumption × dietary metabolizable energy

Voluntary food intake (%) = (mean food consumption × 100)/mean body weight

Feed efficiency = mean body weight gain (g)/total food consumption (g).

2.3. Statistical analysis

The results are presented as means ± standard deviations (SD). Statistical comparisons were performed by two-way ANOVA analysis of variance (two factors: MSG administration and quercetin treatment) complemented by Tukey’s test. Statistical significance was set at p < 0.05. Sigma Plot version 11.0 was used for graphic design and statistics.

3. Results

3.1. Nutritional parameters

As shown in Table 1, MSG-treated rats had increased BW gain while quercetin treatment reduced efficiently BW by about 23%. Food consumption, energy intake and glucose levels were significantly (p < 0.05) higher in MSG-treated rats and became reduced after quercetin administration. Fat acid levels tended to be elevated after MSG-quercetin combination compared to MSG group (Table 1).

3.2. Glucose and lipid metabolism

All experimental groups responded to the OGTT showing increased glucose levels after 30 and 60 min. After 120 min, the glycemic profile of MSG group was not reestablished to basal levels and was higher than CT group. Quercetin administration in MSG-treated rats reduced significantly glucose levels when compared to MSG group (Fig. 1).

The levels of TG, TC, VLDL-cholesterol and LDL-cholesterol were higher (p < 0.05) after MSG administration (Fig. 2). Animals receiving quercetin had significantly reduced TG and VLDL-cholesterol compared to those MSG-QC group. Comparing MSG and MSG-QC groups, quercetin normalized HDL-cholesterol levels and depressed TG, TC, VLDL-cholesterol and LDL-cholesterol levels (Fig. 2).

3.3. Hormone assay

Serum insulin and leptin concentrations were significantly higher (p < 0.05) in MSG group than CT group. However, quercetin treatment had reduced the insulin and leptin levels after MSG administration by about 20% and 45%, respectively (Fig. 3A and B).
3.4. Serum enzymes determination

In addition, MSG group exhibited increased activities of ALT, AST, LDH, ALKP, amylase and creatinine levels, whereas total protein, GSH-Px, SOD and urea were reduced compared to untreated rats (Table 2). Notably, quercetin treatment oppositely decreased ALT, AST, LDH, ALKP, amylase and creatinine levels and also promoted an augment of GSH-Px and SOD activities (Table 2).

4. Discussion

The regulation of energy balance is essential to maintain the control of metabolism and body composition (Scharrer, 1999). Importantly, MSG has been widely used to induce obesity through hypothalamic lesions in neonatal period (Nakayama et al., 2003). Also, Grassioli et al. (2007) have evidenced glucose disturbances only in neonatal MSG-treated animals compared to adult MSG treatment. One of the main causes of hyperglycemia in these rats is related to a decreased amount of GLUT 4 protein found in adipocytes (Macho et al., 2000).

We proposed to investigate the consequences of MSG-induced alterations and the role of quercetin on nutritional and metabolical parameters. The MSG administration increased food and energy consumption and glucose levels after 72 days of age. In addition,
quercetin treatment significantly reduced BW gain and feed efficiency, also having a tendency in reducing food and energy intake in MSG-treated rats. The term feed efficiency refers to the amount of ingested energy which contributes to body weight gain. Flavonoids have been described as modulators of lipid homeostasis in the adipose tissue and liver, through the inhibition of phosphodiesterases (Peluso, 2006). One of the mechanisms of polyphenols actions has been attributed to their antioxidant action resulting in the inhibition of LDL oxidation, but another role for these compounds includes alteration of hepatic cholesterol absorption, triglyceride assembly and secretion (Rivera et al., 2008). Since these alterations might be considered as a problem of defective energy balance regulation (Reeds et al., 1996) and after analyzing dietary effects on energy balance and food-seeking behavior, the potential involvement of the hypothalamic centers must be recognized. The development metabolic impairments has been tightly linked to metabolic alterations, as could be seen by the reductions in TG, impaired tissue insulin sensitivity and/or stimulation of hepatic gluconeogenesis. Although the mechanisms causing metabolic alterations are still obscure, it has been proposed that metabolic dysfunction is associated with deterioration of pancreatic β-cells activity (Prentki and Nolan, 2006). Thus, the enhanced glucose-induced insulin releasing contributes to hyperinsulinemia (Grassioli et al., 2007). Curiously, insulin oversecretion and alteration in pancreatic autonomic nervous system have been reported in these MSG-obese rodents (Balbo et al., 2002).

Most studies have investigated the wide range of concentration by which quercetin (1–80 mg/kg/day) may exert their function. Our protocol is adapted from that previously described by Mahesh and Menon (2004) who tested the effects of quercetin at doses of 50 and 80 mg/kg/day. In contrast to other studies, we found that only doses of 75 mg/kg quercetin, during 42 days, were able to ameliorate all evaluated parameters in a model of MSG-induced metabolic alterations, as could be seen by the reductions in TG, TC, VLDL-cholesterol and LDL-cholesterol levels. These protective effects can be mainly attributed to the antioxidant properties of quercetin (Arai et al., 2000), indirectly acting on the cholesterol levels. Therefore, LDL-chol and VLDL-chol would be protected against deleterious effects arising from oxidative process and its recognition by target cells would be determined (Carrero et al., 2007). Curiously, insulin oversecretion and alteration in pancreatic autonomic nervous system have been reported in these MSG-obese rodents (Balbo et al., 2002).
assembly and secretion as well as through inhibition of phosphodiesterases in the adipose tissue and liver (Peluso, 2006; Rivera et al., 2008). These events could, at least partially, explain the great improvement in lipid profile.

It seems true that insulin sensitivity is influenced by the redox state of the organism, whereby oxidative processes may trigger the development of insulin resistance (Evans et al., 2002). Emerging evidence suggests that oxidative processes may trigger the development of insulin resistance (Evans et al., 2002). This can explain the increased serum TG levels in MSG animals. Also in this context, antioxidant activities could be inversely correlated with insulin and leptin resistance. Administration of quercetin enhanced SOD and GSH-Px activities in MSG-treated rats and only doses of 75 mg/kg quercetin were able to reduce both glucose and insulin levels in these rats. It has been successfully demonstrated that quercetin acts as a powerful antioxidant by either scavenging ROS after diffusion into the lipid bilayer membranes or by promoting antioxidant activities (Vieira et al., 2011). Data from the present study confirm these findings. It seems true that insulin sensitivity is influenced by the redox state of the organism, whereby oxidative processes may trigger the development of insulin resistance (Evans et al., 2002). This can explain the increased serum TG levels in MSG animals. Also in this context, antioxidant activities could be inversely correlated with insulin and leptin resistance. Administration of quercetin enhanced SOD and GSH-Px activities in MSG-treated rats and only doses of 75 mg/kg quercetin were able to reduce both glucose and insulin levels in these rats. It has been successfully demonstrated that quercetin acts as a powerful antioxidant by either scavenging ROS after diffusion into the lipid bilayer membranes or by promoting antioxidant activities (Vieira et al., 2011). Data from the present study confirm these findings.

In conclusion, quercetin successfully improves nutritional parameters and ameliorates the metabolic alterations caused by MSG treatment. Moreover, quercetin normalized glucose levels and minimized the MSG-related toxic effects on liver and kidney functions. These effects are associated to the powerful antioxidant properties of quercetin. We encourage a high intake of plant foods, rich in quercetin, as it produces beneficial effects against metabolic disorders.

Conflict of Interest

None declared.

Acknowledgment

We would like to give a special thank to Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (process no. 02/03359-5) for financial support.

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In conclusion, quercetin successfully improves nutritional parameters and ameliorates the metabolic alterations caused by MSG treatment. Moreover, quercetin normalized glucose levels and minimized the MSG-related toxic effects on liver and kidney functions. These effects are associated to the powerful antioxidant properties of quercetin. We encourage a high intake of plant foods, rich in quercetin, as it produces beneficial effects against metabolic disorders.


