Mechanism of Action of Nutraceuticals on Intestine to Ameliorate Glucose Homeostasis: Follow-up Studies by an In Situ Approach

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Abstract

**Background:** Epidemiologic studies and clinical trials have suggested a correlation between dietary polyphenols and prevention of chronic diseases such as diabetes. The current study has been limited to the compounds previously studied by in vivo and in vitro experimental model concerned glucose homeostasis.

**Objective:** Some selected compounds as myricitrin, quercetin, catechin, naringenin, caffeic acid, rutin, fukugetin, hispidulin, kaempferitrin and chlorogenic acid were investigated in sodium-glucose co-transporter activity in rat intestine through an in situ approach, compared with phlorizin, a classical competitive inhibitor of Na+-dependent glucose transport. Methods: For the in situ studies the intestine segments were uploaded with glucose solution, phlorizin and/or compounds and after 30 min the glucose was measured into the respective intestinal segment.

**Results:** Among the substances assayed, myricitrin, quercetin, catechin, naringenin, caffeic acid, rutin and fukugetin significantly reduced the glucose uptake by affecting the SGLT1 transporter activity measured in the presence of phlorizin. It worthwhile mentions that myricitrin at 10mM exhibited a per se inhibitory effect around 90% higher than that observed for phlorizin. Quercetin inhibited the glucose uptake in both concentrations used and exhibited an effect on glucose absorption as good as phlorizin at 10 mM. Catechin and caffeic acid (10 mM) in the presence of phlorizin potentiated the inhibitory effect of this compound on glucose uptake. Moreover, 10 mM naringenin showed a similar inhibitory effect of phlorizin. Additionally, rutin and fukugetin (10 mM) alone or in combination with phlorizin slightly reduced the glucose absorption.

**Conclusion:** Based on these results, myricitrin, quercetin, catechin, naringenin, caffeic acid, rutin and fukugetin are able to regulate glucose absorption by acting in an intestinal target, SGLT1, contributing to ameliorate glucose homeostasis.

Keywords: SGLT1; Glucose absorption; Flavonoids; Caffeic acid; Polyphenols; Glycemia

Introduction

The dietary glucose cannot cross easily the lipid intestinal bilayer of enterocyte. So, specific transporters are required for glucose intestinal absorption. The Na+/glucose co-transporter (SGLT1) at the enterocyte apical membrane transports glucose into the cell against its concentration gradient. Also, a facilitative Glucose Transporter (GLUT2), transports sugars across the basolateral membrane to the blood [1,2]. Some studies show evidences that GLUT2 can also be recruited to the Brush-Border Membrane (BBM) in the presence of high glucose concentration in the lumen and can participate in the intestine glucose absorption process [3].

SGLT1 has high affinity for glucose and transport two sodium ions for each molecule of glucose. It was postulated that the active glucose transport through the intestinal epithelium is driven by the sodium gradient across the membrane, by means of a Na+/glucose co-transport [4]. It is believed that Na+ and glucose transport by the SGLT1 occur by a sequential mechanism in which two Na+ ions from the extracellular side bind to the transporter just before the glucose induces a conformational change in the glucose binding site and increases the transporter affinity for the substrate. Also, together with the Na+ and glucose transport the SGLT1 promotes depolarization at plasma membrane, which can serve as a signal. In addition, in the absence of glucose the SGLT1 can support a Na+ current [4,5]. Many studies suggest that polyphenols such as phlorizin and phloretin decrease intestinal glucose absorption [6]. Phlorizin, a flavonoid glycoside found in apples, is known as a competitive inhibitor of Na+-...
dependent glucose transport across of BBM [7]. Also, phloretin is the aglycone of phlorizin and an inhibitor of intestinal glucose transport mediated by GLUT2 [8].

Polyphenols are secondary metabolites of plants and found largely in fruits, vegetables, cereals and beverages, thus form an integral part of the human diet. Several biological activities and beneficial properties are described for dietary polyphenols and it has suggested that long term consumption of diets rich in plant-foods polyphenols, including phenolic acids and flavonoids, is associated with some protection against a variety of disease states [9,10]. In recent years, the interest in polyphenols as nutraceuticals and supplementary treatments for various aspects of diabetes mellitus has widely increased [11]. It was showed that dietary plant polyphenols and polyphenol-rich products modulate carbohydrate and lipid metabolism, attenuate hyperglycemia, dyslipidemia and insulin resistance, improve adipose tissue metabolism, and can also prevent the development of long-term diabetes complications [12]. However, the mechanism of action of nutraceuticals in the intestine is not completely understood. So, taking it in account and our previous studies concerned with glycemia control by several natural compounds [9,13-16] also deeply discussed in two chapters [2,11], the aim of the present study was to investigate the acute effect of some selected natural compounds (Figure 1) on glucose transporter, SGLT1, by in situ treatment.

Materials and Methods

Chemicals

Phlorizin, caffeic acid, chlorogenic acid and glucose were purchased from Sigma Chemical Company (St. Louis, MO, USA). Ketamine was purchased from AgenerUnião (Embu-Guáçu, SP, Brazil) and xylazine was purchased from Bayer (Leverkusen, Germany).

Plant material

Kaempferitrin was isolated from leave of the Bauhinia forficata Link [17], quercetin, hispidulin and naringenin were isolated from Baccharis pseudotenuifolia [18], catechin was obtained from bark of the Croton celatidifolius Baill [19], fukugetin was isolated from leaves of the Rheedia gardneriana [20], Rutin was obtained from Polygalta paniculata [21] and myricitrin was isolated from leaves of the Eugenia uniflora [22].

Animals

Male Wistar rats (180–200g) were bred in animal facility and housed in an air-conditioned room (approximately 20 ± 2°C) with a controlled 12: 12 h light/dark cycle (lights on from 06: 00 to 18: 00 h). The animals were maintained with pelleted food (Nuvital, Nuvilab CR1, Curitiba, PR, Brazil) and tap water was available ad libitum. Fasted animals were deprived of food for at least 16 h but allowed free access to water. All the animals were monitored and maintained in accordance with the ethical recommendations approved by the Committee for Ethics in Animal Research of UFSC (Protocol CEUA n° PP00398). For glucose absorption, briefly, rats received anesthesia (ketamine/xylazine; 75/10 mg/kg) association according Gonzalez-Mujica et al. [23].

Glucose intestinal absorption studies

For each in situ experiment, the intestine was exposed and divided in four segments of 4 cm each one by ligatures (Scheme 1). So, 1mL of glucose solution 10 mM was administered by a syringe in each divided portion (segment 1, 2, 3 and 4). Also, in some segments, glucose solution in the presence (segment 2 and 4) and absence of 1 mM of phlorizin (segment 1 and 3) were used. For treated groups, a glucose solution plus compounds in the absence (segment 3) and presence (segment 4) of phlorizin were assayed. Following 30 min the content of each segment was collected and glucose was measured by glucose-oxidase method [24]. Any significant change in volume collected was observed compared with the initial volume added. The assays were carried out according Gonzalez-Mujica et al. [25].
between the groups. Differences were considered to be significant at $p \leq 0.05$.

**Results**

**Time-course of basal glucose absorption**

As it can be seen in Figure 2A, the basal glucose absorption (10 mM) was significantly increased in time-dependent manner from 5 to 30 min. Phlorizin (1 mM) at 30 min was able to block significantly the glucose absorption (Figure 2B). Also, some experiments with 10, 20, 30, 40 and 50 mM of glucose in the presence or absence of catechin and naringenin (10 mM) with/without phlorizin (1 mM) were carried out in order to evaluate the profile of glucose absorption (data not shown). From those data, 10 mM of glucose concentration it was chosen for the next assays since in this condition phlorizin blocked about 100% of glucose absorption.

**Inhibitory effect of nutraceutical compounds on SGLT1**

Surprisingly, 10 mM myricitrin inhibited glucose absorption around 95%. In addition, this compound exhibited 90% of inhibitory effect of phlorizin on glucose absorption (Figures 3A and 3B). On the other hand, 1 mM myricitrin did not alter the glucose absorption compared with control group.

Figures 4A and 4B shows the inhibitory effect of quercetin in both concentrations used. As it is observed, this flavonoid (10 mM) was as good as phlorizin on glucose absorption inhibition.

In addition, 10 mM catechin, an aglycone flavonoid widely present on the diet, inhibited efficiently the glucose absorption by acting on SGLT1 Na⁺-glucose co-transporter on intestine. Beyond, the catechin presented a per se inhibitory effect on glucose absorption and also potentiated the well-known phlorizin effect on intestinal SGLT1. However, any alteration on glucose absorption was observed at 1 mM catechin (Figures 5A and 5B).

Figure 6 shows the in situ effect of naringenin on intestinal Na⁺-glucose cotransporter. As it was expected, phlorizin significantly reduced the glucose absorption about 85% after 30 min of in situ exposition. Also, the administration of 1 mM naringenin not changed the glucose absorption (Figure 6A). However, 10 mM naringenin was able to inhibit glucose absorption around 74% (Figure 6B). It worth mention that naringenin prevented the complete inhibitory effect of phlorizin on glucose absorption.

Figures 7A and 7B show the in situ effect of caffeic acid on glucose absorption after an acute exposition. Surprisingly, this compound showed an inhibitory effect on glucose transporter in both concentrations tested as much efficient as that exhibited by phlorizin at the same experimental condition. In addition, the 10 mM caffeic acid potentiated the inhibitory effect of phlorizin bypassing the maximum effect of phlorizin on inhibition of glucose absorption measured in its experimental approach. Furthermore, rutin and fukugetin (10 mM) alone or in combination with phlorizin reduced slightly the glucose absorption and also both flavonoids reduced phlorizin inhibitory effect (Figures 8A, 8B and 9).

**Figure 1: Chemical structures of the compounds used in this study**
Hispidulin, kaempferitrin, and chlorogenic acid already demonstrated to reduce glycemia in different experimental conditions [15,25], were assayed in situ. However, all of them did not modify the glucose absorption at any dose evaluated (data not shown).

**Discussion**

The functional integrity of intestine is an important factor to the efficient absorption of glucose and its distribution to other tissues via circulation [26]. As glucose is of hydrophilic nature it is not able to readily diffuse through the plasma membrane. So, two main kinds of protein transporters participate to this sugar transport: facilitative transporters that are variable and approximately proportional to water absorption (glucose carriers) and active transporter proteins that are constant and not associated with water absorption (glucose carriers – SGLTs) [2,27].
Figure 4A: Effect of quercetin on intestinal glucose absorption in rat. Measured after 30 min; 10 mM Glucose (a), 10 mM glucose plus 1 mM phlorizin (b), 1 mM quercetin (c), 1 mM phlorizin plus 1 mM quercetin (d). Values are means ± S.E.M.; n=4 animals in each group. *p< 0.05 and **p< 0.001 compared with control group (a)

Figure 4B: Effect of quercetin on intestinal glucose absorption in rat. Measured after 30 min; 10 mM Glucose (a); 10 mM glucose plus 1 mM phlorizin (b), 10 mM quercetin (c), 1 mM phlorizin plus 10 mM quercetin (d). Values are means ± S.E.M.; n=4 animals in each group. *p<0.05, **p<0.01 and ***p<0.001 compared with control group (a)

Figure 5A: Effect of catechin on intestinal glucose absorption in rat. Measured after 30 min; 10 mM Glucose (a); 10 mM glucose plus 1 mM phlorizin (b), 1 mM catechin (c), 1 mM phlorizin plus 10 mM catechin (d). Values are means ± S.E.M.; n=4 animals in each group. ***p< 0.001 compared with control group (a)

Figure 5B: Effect of catechin on intestinal glucose absorption in rat. Measured after 30 min; 10 mM Glucose (a); 10 mM glucose plus 1 mM phlorizin (b), 10 mM catechin (c), 1 mM phlorizin plus 10 mM catechin (d). Values are means ± S.E.M.; n=4 animals in each group. ***p<0.001 compared with control group (a), ###p<0.001 in relation to phlorizin group

The structural diversity of polyphenols makes the estimation of their content in food difficult. However, attempts to estimate the polyphenol intakes have been reported [29-31]. The mean and median of polyphenol intakes for the whole population is around 1.2 and 1.12 g/d [32] and the concentration in plasma rarely exceeds 1 µM after the consumption of 10–100 mg of a single phenolic compound [29].

From our results, myricitrin seems to be a powerful inhibitor of SGLT1 activity and also has alternative sites of action in intestine. Interestingly, these results are additive with antidiabetic effect of myricitrin by acting on aldose reductase and on glucose uptake in C2C12 myotubules under normal and insulin-stimulated conditions [33], suggesting a relevant role of myricitrin on glycemia homeostasis.

In our experimental conditions using 10mM of glucose, phlorizin effectively blocked the glucose absorption indicating that SGLT1 is inhibited. The following experiments were based on the influence of phlorizin (a specific inhibitor of SGLT1) on glucose absorption in the absence (control) or presence of some selected compounds. The inhibitory effect of phlorizin on intestinal glucose absorption was corroborated with those reported in the literature [23,27].

Although the evaluation of polyphenol dietary intake still lacks precision, the dietary intake of naringenin are reported to be about 0.2-0.5 for women and 0.18-0.55 g/d [39]. In our in situ approach studied, naringenin (both concentration assayed), significantly reduced intestinal glucose absorption by around 74% compared with glucose control group and similar to that observed for phlorizin. Furthermore, with both compounds together, the glucose absorption...
almost disappeared suggesting a competition between them with the site of SGLT1.

These data are in line with those reported for naringenin in rabbit and rat intestinal Brush Border Membrane Vesicle (BBMV) when compared with phlorizin. Additionally, the inhibitory effect of naringenin on glucose absorption compared with the powerful action of phlorizin is in accordance with those reported for per se inhibition of naringenin on glucose absorption reported by Li et al. [27].

Quercetin, the main flavonol in our diet (present 0.3 mg/g in onions and 10-25 mg/L in tea) [34,35], showed an in situ inhibition as good as phlorizin on glucose absorption. These data are in agreement with those reported to quercetin on serum glucose lowering in hyperglycemic rats when this compound was administered by oral gavage [15]. Also for quercetin, it is reported that quercetin-3-O-glucoside did not affect SGLT1 and aglycone quercetin regulates glucose absorption by inhibition of GLUT-2 dependent uptake [36].

One of the most abundant flavanol (1g/L) present in the infusion of green tea [37], catechin, beyond inhibit significantly the glucose uptake also potentiated the inhibitory effect of phlorizin on intestinal SGLT1 transporter and also catechin seems inhibit other hexoses transporters since its effect was greater than per se action of phlorizin. As described by Kobayashi et al. [38], catechin (1 mM) did not show significant inhibition on SGLT1. However, the potentiated effect of phlorizin by catechin seems to be mediated by other targets since it was reported that the catechin structure increased the accessibility for another polyphenols residues to the glucose/Na\(^+\)-binding site in SGLT1 [38].
The most frequently phenolic acids encountered in foods are caffeic acid and also is found in the form of esters as chlorogenic acid. Caffeic acid [3,4-di(OH)-cinnamate], found in many types of fruit and coffee in high concentrations [29], exhibited an effective action on reduction of glucose uptake in intestine.

![Figure 8A](image1)

**Figure 8A:** Effect of rutin on intestinal glucose absorption in rat. Measured after 30 min; 10 mM Glucose (a); 10 mM glucose plus 1 mM phlorizin (b), 1 mM rutin (c), 1 mM phlorizin plus 1 mM rutin (d). Values are means ± S.E.M.; n=4 animals in each group. ***p<0.001 compared with control group (a)

![Figure 8B](image2)

**Figure 8B:** Effect of rutin on intestinal glucose absorption in rat. Measured after 30 min; 10 mM Glucose (a); 10 mM glucose plus 1 mM phlorizin (b), 10 mM rutin (c), 1 mM phlorizin plus 10 mM rutin (d). Values are means ± S.E.M.; n = 4 animals in each group. **p<0.01 and ***p<0.001 compared with control group (a), ##p<0.01 in relation to phlorizin group

The role of caffeic acid on diabetes is reported in the literature by attenuating hepatic glucose output, increasing glucose uptake in adipocytes by stimulating insulin secretion. In a whole, caffeic acid suppresses the diabetes states [40].

Furthermore, we observed a similar profile of caffeic acid, myricitrin and catechin since both compounds potentiated the inhibitory effect of phlorizin on glucose uptake. Taking these results together, SGLT1 seems to be a target for some compounds that can be physiologically important to regulate glucose homeostasis during food intake.

It was observed for rutin and fukugetin a slight competitive effect with phlorizin on glucose uptake since both of them inhibited the phlorizin effect. Rutin is known to decrease glycemia by acting in multiple tissues involved on glucose homeostasis [15,41,42]. Taking these data together, it reinforces the effective role of rutin as a candidate to possible application in controlling hyperglycemia and warrants further investigations. As far as we know, for fukugetin it is the first report concerning intestinal SGLT1 transporter as a target for this flavonoid.

![Figure 9](image3)

**Figure 9:** Effect of fukugetin on intestinal glucose absorption in rat. Measured after 30 min; 10 mM Glucose (a); 10 mM glucose plus 1 mM phlorizin (b), 10 mM fukugetin (c), 1 mM phlorizin plus 10 mM fukugetin (d). Values are means ± S.E.M.; n=4 animals in each group. *p<0.05 and ***p<0.001 compared with control group (a), ##p<0.01 in relation to phlorizin group

On the other hand, kaempferitrin already described by our group as antihyperglycemic agent by reducing serum glucose levels [15], in this in situ approach it does not changed glucose uptake. Also, hispidulin and chlorogenic acid also did not affect the SGLT1 activity. So, it is clear that the intestinal glucose transporters are not the main target for these compounds to regulate glucose homeostasis (data not shown). Other studies suggest that chlorogenic acid presents a hypoglycemic effect and might have an antagonistic effect on glucose transport [43,25]. However, for hispidulin only a report concerned its antidiabetic effect is available in the literature and the details concerned the mechanism of action is not available [44].

**Conclusions**

Taking together these data, we conclude that some selective nutraceutical compounds as myricitrin, quercetin, catechin, naringenin, caffeic acid, rutin and fukugetin are able to regulate glucose absorption by acting in an intestinal target, SGLT1, measured by in situ approach. In a whole, these data indicates that such compounds bind to the glucose transporter and antagonize the glucose transport similar to phlorizin. Studies are underway in order to elucidate the presence of these compounds also on sodium ionic balance in order to inhibit glucose absorption in intestine.

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