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Analytical Methods

Analysis of chlorogenic acids in beverages prepared from Chinese health foods and investigation, *in vitro*, of effects on glucose absorption in cultured Caco-2 cells

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Abstract

The effects of aqueous extracts of Kuding tea (*Ilex latifolia* Thunb.) and the large-leaf form of *Camellia sinensis* var. *sinensis*), chrysanthemum (*Dendranthema morifolium* Ramat), honeysuckle flower (*Lonicera japonica* Thunb.), and purple sweet potato (*Ipomoea batatas*) stem on glucose absorption were investigated using Caco-2 cells. Glucose absorption by Caco-2 cells was significantly inhibited by aqueous extract of Kuding tea, chrysanthemum and purple sweet potato stem under both Na^+-dependent conditions and Na^+-free conditions indicating effects on SGLT1 and GLUT transporters. Analysis of the (poly)phenols in these aqueous extracts suggested that dicaffeoylquinic acids and flavanols may be particularly important in producing these effects. Kuding tea extract was the most effective, suggesting that this merits evaluation in a clinical study.

Keywords: Caco-2 cells; *Camellia sinensis*; Chlorogenic acids; Chrysanthemum; *Dendranthema morifolium*; Flavanols; Glucose absorption; Glucose transport; GLUT; Honeysuckle; *Ilex latifolia*; *Ipomoea batatas*; Kuding; SGLT1; Sweet potato

1. Introduction

Kuding (*Ilex latifolia* Thunb.), chrysanthemum (*Dendranthema morifolium* Ramat), honeysuckle (*Lonicera japonica* Thunb.), and purple sweet potato (*Ipomoea batatas*) are consumed as beverages and vegetables in China where their consumption is believed to provide health benefits. For example, it has been reported that triterpenoids from Kuding have inhibitory effects on acyl CoA cholesterol acyl transferase, thus potentially protecting against arteriosclerosis and obesity (Negishi, Negishi, Yamaguchi, & Sugahara 2004), that chrysanthemum has been used to cure 'liver-fire' and treat eye disease (Anonymous, 1985), and that honeysuckle has antipyretic properties (Anonymous, 1985). In contrast, there have been few studies on the physiological and pharmacological effects of consuming sweet potato. The phytochemical composition of sweet potato has been recently reported (Wang & Clifford, 2008), revealing the presence of chlorogenic acids (Clifford, Wu, Kirkpatrick, & Kuhnert, 2007; Wang & Clifford, 2008), flavanols (Liang, Xu, Hu, & Liu, 1992) and other flavonoids (Liu, Xu, Liang, & Hu, 1992). The most widely occurring chlorogenic acid and one of the few that is available commercially, 5-caffeoylquinic acid, is a competitive inhibitor of hepatic glucose 6-phosphatase (Arion et al., 1997) but the extent to which 5-caffeoylquinic acid reaches the liver unmetabolised *in vivo* is unclear.

5-caffeoylquinic acid is found in abundance in coffee beans and commercial coffee products. Recently, several independent studies in Europe (Rosengren, Dotevall, Wilhelmsen, Thelle, & Johansson, 2004; Tuomilehto, Hu, Bidel, Lindstrom, & Jousilahti, 2004; van Dam & Feskens, 2002) have indicated that greater coffee consumption is

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associated epidemiologically with a reduced risk of developing type 2 diabetes mellitus. The substances and mechanism(s) responsible remain uncertain, though there is some evidence from *in vitro* studies that 5-caffeoylquinic acid might dissipate the Na^+ electrochemical gradient which provides the driving force for active absorption of glucose (Welsch, Lachance, & Wasserman, 1989).

More recently, Johnston, Clifford, and Morgan (2003) have demonstrated that coffee consumption by volunteers delayed the absorption of glucose. Indeed, many dietary (poly)phenols have been shown, at least *in vitro*, to modulate glucose uptake. Green tea polyphenols such as (–)-epigallocatechin gallate (EGCG) and (–)-epicatechin gallate (ECG) also inhibit the Na^+-dependent glucose transporter (Hossain et al., 2002; Kobayashi et al., 2000) and the Na^+-independent facilitative transporter (Johnston, Sharp, Clifford, & Morgan, 2005). Some quercetin glucosides inhibit glucose uptake into brush-border-membrane vesicles of porcine jejunum (Cermak, Landgraf, & Wolffram, 2004) and Gee, DuPont, Rhodes, and Johnson (1998) have shown that some quercetin glucosides are capable of interacting with the Na^+-dependent glucose transporters in the mucosal epithelium and, as a consequence, this may even be the route by which these flavonol glucosides are absorbed by the small intestine *in vivo* (Gee et al., 1998).

In this study we examined aqueous extracts of plants rich in various (poly)phenols for their ability to inhibit glucose uptake by Caco-2 cells as a screening procedure to identify beverages which might merit further investigation in clinical studies.

2. Materials and methods

2.1. Materials

Kuding tea bags were purchased from the tea product factory in Hunan Agricultural University. These are a specially-developed product containing a mixture of Kuding (*I. latifolia* (Thunb.)) and the large-leaf form of *Camellia sinensis* var. *sinensis* grown characteristically in Yunnan Province, China, an unusual form of green tea where the dominant flavanol is (–)-epicatechin gallate (Shao, Clifford, & Powell, 1995; Shao, Powell, & Clifford, 1995). Purple sweet potato stem (*I. batatas*) was collected from Hunan Agricultural University; chrysanthemum (*Dendranthema morifolium* cv. *Gonju*) produced in Hanhui province, China, was purchased from a tea store in Changsha, China, and honeysuckle flower (*L. japonica* Thunb.) was collected from Zhang jiajie, Hunan province, China. All samples were freeze-dried and the dry material stored at 4 °C.

5-Caffeoylquinic acid and heat-inactivated fetal bovine serum were purchased from Sigma Chemical Company (Poole, UK). Cynarin (1,3-dicaffeoylquinic acid) was obtained from LGC Promochem (Hatfield, UK). D-[6-^{3}H] glucose was supplied by Amersham Pharmacia Biotech UK Ltd, Buckinghamshire, UK. Cell culture medium and plasticware were purchased from Life Science Technologies (Paisley, UK) unless stated otherwise. Methanol and acetonitrile for HPLC were purchased from Fisher (UK). All other chemicals were of the highest grade available and bought from reputable commercial sources.

2.2. Preparation of plant extracts

To imitate the method commonly used to prepare beverages for human consumption, 1.5 g of freeze-dried plant material were soaked in 250 ml of boiling water and this suspension incubated for 60 min in a capped thermos flask. The insoluble material was removed from the extract by filtration with a Whatman No. 1 filter paper and 0.5 ml Carrez A reagent added to the filtrate, the mixture vortexed for 20 s and allowed to stand for 1 min. This procedure was repeated with Carrez B reagent and the mixture centrifuged at 2000*g* for 20 min. The aqueous supernatants were tested for effects upon glucose uptake by cultured Caco-2 cells.

2.3. Cell culture

Stock cultures of Caco-2 TC7 cells were maintained in 25 cm^2 plastic flasks and cultured in a 95% air/5% CO_2 atmosphere in Dulbecco's modified Eagle's minimal essential medium (DMEM), supplemented with 20% heat-inactivated fetal bovine serum, 1% penicillin/streptomycin, 1% non-essential amino acids and 1% L-glutamine. All experiments were carried out on cells between passage numbers 30 and 40. For experiments, cells were seeded at a density of 1×10^4 cells/cm^2 into six-well plate inserts (Costar UK, Buckinghamshire, UK) and were grown for 19–21 days.

2.4. Measurement of glucose uptake by Caco-2 TC7 cell monolayers

Glucose uptake assays were performed using HEPES-buffered salt solution (HBSS, pH 7.5: 140 mM NaCl, 5 mM KCl, 1 mM Na_2HPO_4, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 10 mM HEPES) containing 1 mM glucose and a 1 in 10 dilution of the plant extracts. D-[6-^{3}H] Glucose was used as the tracer and the final amount of radioactivity in the control or test solutions was 125 kBq/ml. When a sodium-free buffer was required for investigating facilitative transport, NaCl and Na_2HPO_4 in HBSS were replaced with equimolar amounts of KCl and K_2HPO_4, respectively.

Caco-2 cells were placed in serum-free media for 24 h prior to uptake studies and were incubated for 15 min at room temperature in HBSS prior to commencing experiments. Uptake was initiated by the addition of either the control or test solutions and the reaction was terminated after 2 min by aspiration of the uptake buffer, followed by the addition of ice-cold PBS. Cells were washed twice more in ice-cold PBS and solubilised overnight in 200 mM NaOH prior to scintillation counting.