A kinetic model for in-vitro intestinal uptake of L-tyrosine and D (+)-glucose across rat everted gut sacs in the presence of Momordica charantia, a medicinal plant used in traditional medicine against diabetes mellitus

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Abstract

Momordica charantia (MC) is a traditional antidiabetic medicinal plant used in many parts of the world, including Mauritius. An everted rat gut sac technique was used to investigate the effect of MC on kinetic parameters of D (+)-glucose and L-tyrosine. Everted guts were mounted in a gut sac bath and aqueous extract of MC fruit was added to the mucosal medium (3.62 mg/mL) at varying substrate concentrations. Michaelis-Menten constant (Kₘ) and maximal velocity (Vₘₐₓ) were calculated in the presence and absence of MC fruit extract. It was observed that MC significantly reduced Vₘₐₓ of D(+)-glucose uptake by 0.09 mM hr⁻¹, whereas Kₘ remained unaltered suggested a non-competitive type of inhibition was present. L-Tyrosine uptake in the presence of MC fruit extract did not fit to a relatively simple kinetic model.

Key words: Momordica charantia, kinetics, diabetes mellitus, traditional medicine

Diabetes mellitus’a karşı kullanılan geleneksel tıbbi bitki Momordica charantia varlığında ters yüz edilmiş sıçan bağırsakdan L-tirozin ve D(+)glukoz geçişi için in vitro kinetik model

Özet

Momordica charantia, (MC) Mauritius’da dahlı dünyanın birçok yerinde geleneksel antidiyabetik tıbbi ilaç olarak kullanılmaktadır. Ters yüz edilmiş bağırsak tekniği MC nin D(+)glukoz ve L-tirozinin kinetik parametrelerine olan etkilerini araştırmak için kullanıldı. Ters yüz edilmiş bağırsaklar bağırsak banyosuna kondu ve değişik konsantrasyonda substrat içeren mukoza medyumuna MC meyvesinin sulu ekstresi eklendi (3.62mg/mL). Michaelis-Menten sabit (Kₘ) ve maksimum hız (Vₘₐₓ) MC meyve ekstresinin mevcudiyetine ve yokluğunda hesap edildi. MC nin kayda değer bir şekilde D(+)glukoz alımı Vₘₐₓ’i azalttı (0.09 mM hr⁻¹), buna karşın Kₘ’nin değişmediği ve bunun da rekabet etmeyen bir inhibisyon tipini ortaya koyduğu belirlendi. MC meyve ekstresi varlığında L-tirozin alımı nisbeten basit olan kinetik modele uyguluk göstermedi.

Anahtar sözcükler: Momordica charantia, kinetik, diyabet, geleneksel tıbbi
Introduction

*Diabetes mellitus* (DM) is a debilitating and often life-threatening disease with increasing incidence in rural populations throughout the world. It was postulated that DM is the most common chronic disorder affected more than 176 million people worldwide, and this global figure has been set to double by the year 2030 (Tiwari and Madhusudana, 2002). DM does not only kill, but also is one of the major causes of adult blindness, kidney failure, gangrene, neuropathy, heart attack, and stroke (Bransome, 1992). In Mauritius, DM is becoming a devastating scourge with more than 100,000 cases and with 4.6% death rate. Nephritic syndromes, nephrosis and amputations were prominent prior to the deaths in 2000 (Ministry of Health & Quality of Life, 2003).

Before the introduction of insulin in 1922, the treatment of DM relied heavily on dietary measures, which included the use of traditional plant therapies (Alison and Flatt, 1998). The Papyrus Ebers of 1550 BC had recommended a high-fiber diet of wheat grains and orche (Bailey and Day, 1989). More than 1200 species of organisms have been used ethnopharmacologically or experimentally to treat symptoms of DM, and several reviews on plants with known antidiabetic activity or with traditional use as antidiabetic remedies have been published (Fransworth and Segelman, 1971; Agganonkar, 1979; Oliver-Bever, 1980; Bailey and Day, 1988; Winkelman, 1989). They described more than 725 genera in 183 families, extending phylogenetically all the way from marine algae and fungi to advanced plants such as composites (Marles and Farnsworth, 1995). It would thus appear that traditional antidiabetic plants might provide a useful source for developing new oral hypoglycemic compounds as pharmaceutical entities or simple dietary adjuncts to the exiting therapies. Although an orally active botanical substitute to endogenous insulin seems unlikely, developing new phytochemical molecules, which may stimulate endogenous insulin biosynthesis and secretion (and promote insulin action) is a realistic possibility (Bailey and Day, 1998). In other words, as several investigators suggested, studying such traditional medicines might offer an alternative and natural key to unlock diabetologists’ pharmacy.

On the other hand, suggested mechanisms describing therapeutic effects of several traditional medicinal plant systems are holistic (Handa et al., 1989). Most of the reported hypoglycemic plants are anecdotal, and only few have received adequate scientific evaluation. The fundamental mechanisms of these medicinal systems are still unexplainable using modern tools (Rahman and Zaman, 1989). It is claimed that most medicinal preparations in traditional medicines contain a variety of synergistically acting phytochemicals that are thought to act on a variety of targets by various modes and mechanisms (Tiwari and Madhusudana, 2002).

In accordance with the recommendation of the World Health Organisation expert committee on DM (Alison and Flatt, 1991), investigation of hypoglycemic agents from plants, which have been used as traditional medicines seems of paramount importance. In this study, we investigated the effect of *Momordica charantia* (MC), a well-documented hypoglycemic plant (Chatterjee, 1994; Singh, 1986; Ng et al., 1986), on the kinetic uptake of D(+)-glucose and L-tyrosine in *in-vitro* everted gut sac model as described by Subratty (2003).

Materials and methods

**Preparation of the crude extracts from Momordica charantia fruit**

A powdered mixture of MC dry fruit (10g) was extracted with 50 mL water at 90°C for 5 hours using Soxhlet apparatus. The water was evaporated under vacuum at 50°C, and the precipitate was transferred into 10 mL distilled water. Percentage yield was calculated, and the paste-like material was diluted in distilled water to use in experiments.

**Experimental design and surgical procedure**

Adult male Swiss albino rats weighing 100-150 g and housed at temperature 25 ± 2°C were used in this study. Animals were maintained on commercial feed and tap water *ad libitum*. Before each experiment, the animals were starved for 12 hours but allowed for tap water *ad libitum* use. Rats were sacrificed by severe blow on the head against a hard surface. The abdomen was opened by a midline incision. The entire small intestine was removed quickly by cutting across the upper end of the duodenum and the lower end of the ileum, and by stripping the mesentery manually (Barthe et al., 1998). The small intestine was then washed out with normal saline solution (0.9% w/v NaCl) using a syringe equipped with blunt end.
Preparation of everted gut sacs

Intestinal segments (10±2 cm) were everted according to the method described by Wilson & Wiseman. After being blotted with a piece of filter paper, a 1 g glass weight was fixed and tied to the end of the everted gut segment to make an empty gut sac. This was important to prevent peristaltic muscular contractions, which may otherwise alter the shape and internal volume of the sac. The 1 g glass weight was the minimum weight to secure the above-mentioned conditions and to prevent the sac septum to become thin. A scheme describing the gut sac bath system used in the study was shown in Figure 1.

After weighing, the empty sac was filled with 1 mL of Krebs-Henseleit bicarbonate buffer (KHB). The composition of the buffer was (mM/L): NaHCO₃ 25; NaCl 118; KCl 4.7; MgSO₄ 1.2; NaH₂PO₄ 1.2; CaCl₂ 1.2; and Na₄EDTA 9.7 mg/L. Glucose (2g/L) was added to the medium just before the start of the appropriate experiment. The pH was maintained at 7.4. The sac was filled with a blunted-ended syringe and then the needle was slipped off carefully, and the loose ligature on the proximal end was tightened. The filled and distended sac was weighted and the difference in weight was taken as the measure of the initial serosal volume. The compartment containing the buffer in the sac was named serosal fluid compartment.

The distended sacs was placed inside a 50 mL KHB bath (mucosal fluid compartment) and mounted as described by Khoshapur and Chaideh (1999). This gut sac bath was surrounded by a water jacket maintained at 37-40 °C. The mucosal fluid compartment was continuously mixed with air bubbles using the mixture of 95 % O₂ and 5% CO₂, and pH maintained at 7.5.

Effects of Momordica charantia on the uptake of glucose and tyrosine transport

For studying the effect of the plant extract on the uptake of glucose and tyrosine (substrates), glucose and tyrosine at varying concentrations were added into mucosal compartment fluid. The plant extract was also added in the same compartment (3.62 mg/mL).

Measurements of final volume

At the end of the incubation period (30 min), the sacs were removed from the gut sac bath, blotted by a standardized procedure as described above and weighted. The serosal fluid was drained through a small incision into a test tube. The emptied sac was shaken gently to remove the adhered fluid and the tissue was weighted. The final serosal volume was determined by subtracting (after incubation) the weight of the empty sac from that of the filled sac. The mucosal fluid transfer was expressed in the terms of diminution of fluid volume in the mucosal compartment during the course of experiment. The serosal fluid transfer was reflected as an increase in the volume of serosal compartment inside the sac. The gut fluid uptake was determined by measuring an increase in the volume of fluid in the gut wall.

Tyrosine concentrations in both compartments were determined by a spectrophotometric method (Subratty, 2003). Glucose concentrations were measured using a commercially available glucose oxidase kit (Boehringer Mannheim, Mannheim, Germany). The amount of tyrosine and glucose
transported from the mucosal compartment was characterized as 'uptake' while the serosal gain of the substances is treated as 'release'. Uptake and release of glucose and tyrosine were expressed as mM/g tissue wet weight/h. All chemicals were procured from Sigma (UK). Glucose and tyrosine experiments were performed separately.

Control experiments

In each serie of experiments, control everted gut sacs derived from the same rat in a buffer containing no substrate were run in parallel. The controls were run either with or without MC and results were corrected accordingly.

Data analysis

In terms of enzyme kinetics, the amount of L-tyrosine and glucose transported per hour were analogue to the velocity of transfer, in other words, to the concentration difference of the tyrosine and glucose between compartments at the beginning and end of an experiment (Subratty, 2003). The Michaelis - Menten constant (K<sub>m</sub>), which is the affinity of the transferring enzyme for the substrate, and maximal velocity (V<sub>max</sub>), which is the rate of transfer reaction, in the presence as well as in the absence of MC were determined from the differences of uptake and release values using the Michaelis-Menten and Lineweaver-Burk Plots in Microsoft Excel 2000. Comparison of difference between the controls and experimental groups were examined using One-Way Analysis of Variance (ANOVA) test for mean ± SEM of K<sub>m</sub> and V<sub>max</sub>. Any difference with p values less than 0.05 were considered as statistically significant. Mean K<sub>m</sub> and V<sub>max</sub> were presented as single entities in tables.

Results

Table 1 lists the biochemical parameters of D (+)-glucose transport across rat everted small intestines in-vitro. The K<sub>m</sub> and V<sub>max</sub> were calculated in the absence as well as in the presence of MC fruit extract in the mucosal solution. Data analysis revealed that V<sub>max</sub> for glucose uptake decreased (P < 0.05) by 0.09 mM hr<sup>-1</sup> in the presence of the aqueous MC fruit extract. In contrast, the apparent K<sub>m</sub> (22.2 Mm) remained unaltered (P>0.05) in the presence of the fruit extract.

Table 1: Biochemical parameters obtained for the effect of MC fruit extract on the transport of D ( +)-glucose at different concentrations (4-10 mM) across the rat everted gut sacs. N= number of sacs used. The everted gut sacs were incubated in Krebs- Henseleit buffer (pH= 7.4) at 37°C.

<table>
<thead>
<tr>
<th>Experiments</th>
<th>V&lt;sub&gt;max&lt;/sub&gt; (mM hr&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>K&lt;sub&gt;m&lt;/sub&gt; (mM)</th>
</tr>
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<tbody>
<tr>
<td>Control (N=7)</td>
<td>0.17</td>
<td>22.2</td>
</tr>
<tr>
<td>Momordica charantia fruit extract (3.62 mg/ml) (N=7)</td>
<td>0.08</td>
<td>22.2</td>
</tr>
</tbody>
</table>

Table 2 depicts the biochemical parameters of L-tyrosine uptake across rat everted small intestines in-vitro. Incubation of gut sacs in MC containing mucosal solutions with varying concentration of tyrosine did not follow a simple kinetic model. Negative values for V<sub>max</sub> (-0.01 mM hr<sup>-1</sup>) and K<sub>m</sub> (-4.0 Mm) were obtained for the tyrosine uptake across the everted gut sacs.

Table 2: Biochemical parameters obtained for the effect of MC fruit extract on the transport of L-Tyrosine at different concentrations (0.5-2.0 mM) across the rat everted gut sacs. N= number of sacs used. The everted intestines were incubated in Krebs-Henseleit buffer (pH= 7.4) at 37°C.

<table>
<thead>
<tr>
<th>Experiments</th>
<th>V&lt;sub&gt;max&lt;/sub&gt; (mM hr&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>K&lt;sub&gt;m&lt;/sub&gt; (mM)</th>
</tr>
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<tbody>
<tr>
<td>Control (N=7)</td>
<td>0.03</td>
<td>0.075</td>
</tr>
<tr>
<td>Momordica charantia fruit extract (3.62 mg/ml) (N=7)</td>
<td>-0.01</td>
<td>-4.0</td>
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Discussion

Progress in understanding the metabolic staging of DM over the past few years has led to significant advances in regimen for treatment of this devastating disease. The most challenging goal in the management of patients with DM is to achieve blood glucose level as close to normal as possible. Unfortunately, post-prandial hyperglycaemia (PPHG) or hyperinsulinaemia are independent risk factors for the development of vascular complications in DM patients (Tiwari and Madhusudana, 2002). Starting from the very beginning
of carbohydrate metabolism, mechanisms playing role in release and transport of glucose across the intestinal brush border membrane down to the blood stream have attracted much attention recently as potential targets to control PPHG. In this category, majority of recent studies reported the potential use of anti-diabetic medicinal plants on inhibition of glucose transport. Drugs that reduce PPHG by suppressing the absorption of carbohydrate are effective in prevention and treatment of non-insulin dependent DM. Recently, Matsuda et al. (1998) studied the effect of plant saponins on the transport of glucose in-vitro.

Our findings would tend to indicate that glucose transport was significantly decreased in the presence of the crude extract of <i>Momordica</i> fruits, which caused a decrease in the $V_{max}$ by 0.09 mM hr$^{-1}$. However, the $K_m$ (22.2 mM) remained unaltered in the presence as well in the absence of the fruit extract (Table 1). This indicates that MC act by bringing a non-competitive type of inhibition of glucose at the level of the small intestine. Therefore, it is most probable that active phytochemicals in the fruit of MC binds on the glucose transporters thus may lead to wash out of glucose from the body. The latter may be responsible for the hypoglycemic phenomena after MC fruit juice was administered noted by various investigators in animals or subjects (Leatherdale et al., 1981; Aktar, 1982). Based on this data generated in this study, regarding the mechanism of action, we propose that MC extracts may possess hypoglycaemic properties by inhibiting the glucose transport at the site of intestinal brush border membranes.

In simple kinetic model, an amino acid ‘A’ interacts with a membrane component or “carrier-site” ‘X’ to form a binary complex. Investigation of the directional influx of tyrosine from the mucosal membranes into the epithelium here indicated that the transfer process did not follow reasonably well the simple kinetic model. The $V_{max}$ and $K_m$ values for tyrosine in the presence of the fruit extract were negative (Table 2), similar to our previous observation (Subratty, 2003). We have proposed that the negative values for the kinetic parameters could be due to reversal of the normal sodium and potassium gradient across the brush border membrane, which in turn translocated tyrosine back to the mucosal surface. Phytochemicals in the extract (saponins and glycosides) may alter sodium-potassium gradient on the membrane. The negative values might mean lack of transfer rather than pumping non-existing tyrosine in serosal compartment back to mucosal compartment, and may arise due to technical limitations. Under these conditions, sodium required to form ternary complex in the relevant binding protein, which otherwise possesses dislocation, may derive from the serosal fluid, and this conformational change in the protein may reverse the transport (Subratty, 2003). However, a clear picture for the mechanism is not clear. Further work needs to be undertaken to investigate the exact nature of possible carriers, which may involve in tyrosine transport in the presence of the fruit extract.

In conclusions, our study provides evidence for a biochemical mechanism which carries blood glucose lowering effect of <i>Momordica charantia</i> fruit in intestine via non-competitive inhibition. However, further kinetic data on carrier-mediated transport of D (±)-glucose and L-tyrosine in the presence of MC fruit extract is needed.

References


Chatterjee KP. On the presence of an antidiabetic principle in <i>Momordica charantia</i>. <i>Ind J Physiol Pharmacol</i>. 7: 240, 1964.


