INHIBITORY MECHANISMS OF OLEANOLIC ACID 3-*O*-MONODESMOSIDES ON GLUCOSE ABSORPTION IN RATS

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We examined the action mechanism of oleanolic acid 3-*O*-monodesmoside, momordin Ic (1), and oleanolic acid 3-*O*-glucuronide (2) for the inhibitory effect on the increase in serum glucose levels in oral glucose-loaded rats. Although 1 and 2 dose-dependently inhibited the increase in serum glucose levels in oral glucose-loaded rats, these compounds showed no significant effects on serum glucose levels in normal rats, intraperitoneal glucose-loaded rats, and alloxane-induced diabetic mice. Furthermore, 1 and 2 were found to suppress gastric emptying in rats, and also to inhibit the glucose uptake in rat small intestine concentration dependently *in vitro*. These results indicate that 1 and 2 given orally have neither insulin-like activity nor insulin releasing-activity. 1 and 2 apparently inhibited glucose absorption by suppressing the transfer of glucose from the stomach to the small intestine and by inhibiting the glucose transport system at the small intestinal brush border.

KEY WORDS oleanolic acid 3-*O*-monodesmoside; momordin Ic; oleanolic acid 3-*O*-glucuronide; gastric emptying; glucose absorption; glucose uptake

Many traditional Chinese and Japanese medicines are known to have preventive and therapeutic effects in diabetes and obesity, but their active components have not yet been characterized except for a few samples. Recently, we have found that the extracts of several natural medicines show inhibitory activity on the increase in serum glucose levels in glucose-loaded rats. Through bioassay-guided separation, we have characterized the active saponin constituents from *Aralia elata* (roots, bark, and young shoots),¹⁾ *Aesculus hippocastanum* (seeds),²⁾ *Beta vulgaris* (roots),³⁾ *Polygala senega* var. *latifolia* (roots),⁴⁾ *Gymnema sylvestre* (leaves),⁵⁾ and *Kochia scoparia* (fruit).⁶⁾ In addition, by examination of the structure requirement for the inhibitory activity on the increase in serum glucose levels, the active saponins could be classified into the following three types of structure: 1) olean-12-en-28-oic acid 3-*O*-monodesmoside; 2) acylated polyhydroxyolean-12-ene 3-*O*-glucuronide; and 3) olean-12-ene 3,28-*O*-acylated bisdesmoside. Among them, olean-12-en-28-oic acid 3-*O*-monodesmosides were found to show the most potent activity. However, the action mechanism of those saponins in their inhibitory activity on the increase in serum glucose levels was left uncharacterized. In this paper, we describe the plausible mechanism of the inhibitory activity by using two olean-12-en-28-oic acid 3-*O*-monodesmosides, momordin Ic (1) and oleanolic acid 3-*O*-glucuronide (2), which were isolated from the fruit of *Kochia scoparia*.⁶⁾

Momordin Ic (1) and oleanolic acid 3-*O*-glucuronide (2) dose dependently inhibited the increase in serum glucose levels in oral glucose-loaded rats (Fig. 1). However, 1 and 2 showed no significant effects on serum glucose levels in normal rats, intraperitoneal glucose-loaded rats, and alloxane-induced diabetic mice, as shown in Fig. 2. These results indicated that 1 and 2 had neither insulin-like activity nor insulin-releasing activity like tolbutamide, and therefore it seemed that 1 and 2 affected glucose absorption in the gastro-intestinal tract. As shown in Fig. 3, 1 and

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Fig. 1. Inhibitory Effect of Momordin Ic (1) and Oleanolic Acid 3-O-Glucuronide (2) on Serum Glucose Levels in Glucose-loaded Rats

Male Wistar rats weighing 130-170 g were fasted for 20-24 h and the test compounds were given orally. Thirty minutes thereafter, glucose (0.5 g/kg) was administered orally. Blood was collected from the jugular vein. Each column represents the mean with S.E. of the changes in serum glucose levels 30 min after glucose administration. Asterisks denote significant differences from the controls at * $p < 0.05$, ** $p < 0.01$ ($n = 5, 6$).

Fig. 2. Effects of Momordin Ic (1) and Oleanolic Acid 3-O-Glucuronide (2) on Serum Glucose Level in Normal and Intraperitoneal Glucose-loaded Rats and Alloxane-induced Diabetic Mice

i) Normal rats: Blood samples were collected 60 min after the administration of the test compound ($n = 4, 5$). ii) Glucose *i.p.*: Glucose (0.5 g/kg) was administered intraperitoneally (*i.p.*). Thirty minutes thereafter, blood samples were collected ($n = 5$). iii) Alloxane (50 mg/kg) was injected into the tail vein. Three days thereafter, each test compound was given orally. Blood samples were collected 60 min after the administration of the test compound ($n = 8$). Asterisks denote significant differences from the controls at ** $p < 0.01$.

2 significantly suppressed gastric emptying in rats. In particular, **1** strongly suppressed gastric emptying at the dosage of 25 mg/kg, while **2** showed significant suppression at 50 mg/kg, although **2** significantly inhibited the increase in serum glucose levels in glucose-loaded rats at the 25 mg/kg, dose as shown in Fig. 1. The potent slowing activity of **1** and **2** on gastric emptying seemed to be important factor in exhibiting inhibitory activity on the increase in serum glucose levels after oral administration of glucose. **1** and **2** also concentration dependently inhibited glucose uptake in rat small intestinal fragments, as shown in Fig. 4. Phlorizin is well known to be an inhibitor of the Na^+ /glucose co-transport system at the intestinal brush border membrane.⁷⁾ Phlorizin also inhibited glucose uptake in a dose-dependent manner (0.001-0.1 mM), but phlorizin showed no more inhibition at a concentration of 1 mM, so the uptake in the presence of high concentrations of phlorizin might be mainly due to nonspecific adsorption or retention of glucose in intercellular spaces. On the basis of the above-mentioned evidence, it was assumed that oleanolic acid 3-O-monodesmosides such as **1** and **2** inhibited glucose absorption by suppressing the transfer of glucose from the stomach to the small intestine, and by inhibiting the active glucose transport system in the small intestine.

Fig. 3. Inhibitory Effect of Momordin Ic (1) and Oleanolic Acid 3-O-Glucuronide (2) on Gastric Emptying in Rats

Test food consisted of 10% glucose, 1% CMC-Na, and 0.05% phenol red was given orally (5 ml/kg) to rats, then the stomach was removed and homogenated. 0.5 ml of 20% TCA was added to the homogenate, and the solution was centrifuged. 0.5N NaOH was added to the supernatant and the amount of phenol red was determined by the absorbance at 560 nm. Each test compound was given orally 30 min before the administration of test food. Asterisks denote significant differences from the controls at $**p < 0.01$ ($n = 5, 6$).

Fig. 4. Inhibitory Effects of Momordin Ic (1) and Oleanolic Acid 3-O-Glucuronide (2) on Glucose Uptake in Rat Small Intestinal Fragments (*in vitro*)

Small fragments (0.1-0.15 g) of everted rat intestine were placed in modified Krebs-Henseleit solution, pH 7.4, with ^{14}C -U-glucose (2 mM, 10^5 CPM/ml). Incubation was carried out at 30°C for 6 min, and washed 2 times for 3-5 s with medium containing 1 mM phlorizin without ^{14}C -U-glucose, and placed on filter paper to absorb the water from the tissue. Tissue was then weighed and dissolved by Soluene 350 (Packard) and the radioactivity was examined. Asterisks denote significant differences from the controls at $**p < 0.01$ ($n = 8$).

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