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Effects of Oleanolic Acid Glycosides on Gastrointestinal Transit and Ileus in Mice

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Abstract—The effects of various oleanolic acid glycosides obtained from medicinal herbs on gastrointestinal transit (GIT) and ileus were investigated in fasted mice. Ileus was induced by the peritoneal-irritation or by the laparotomy with manipulation. One hour after the oral administration, three oleanolic acid 3-*O*-monodesmosides (oleanolic acid 3-*O*-glucuronide (**3**, 50 mg/kg), momordin Ic (**4**, 25 and 50 mg/kg), and momordin I (**6**, 25 mg/kg)) significantly accelerated GIT, but two oleanolic acid 3-*O*-monodesmosides (28-deglucosyl-chikusetsusaponins IV (**8**) and V (**10**)), oleanolic acid 3,28-*O*-bisdesmosides (momordin IIc (**5**), chikusetsusaponins IV (**7**) and V (**9**)), and their common aglycon (oleanolic acid (**1**)) (50 mg/kg) showed no significant effect. On the other hand, oleanolic acid 28-*O*-monodesmoside (compound O (**2**, 50 mg/kg)) significantly inhibited GIT. **4** (5–25 mg/kg) and **6** (12.5 and 25 mg/kg) also significantly prevented the inhibition of GIT induced by the peritoneal injection of acetic acid. **2** and **9** (50 mg/kg) significantly potentiated the inhibition of GIT, whereas **1**, **3**, **5**, **7**, **8**, and **10** (50 mg/kg) showed no significant effect. **3**, **4**, **6**, and **10** (50 mg/kg) significantly prevented the inhibition of GIT induced by laparotomy with manipulation, while **1**, **2**, **5**, **7**, **8**, and **9** (50 mg/kg) showed no significant effect. These results indicate that the 3-*O*-glycoside moiety seems to be essential to show the GIT accelerating activity, and the 28-*O*-glucoside moiety reduce the activity. The accelerations of GIT by **3**, **4**, and **6** were completely abolished by the pretreatment with streptozotocin (100 mg/kg, iv), but not by the pretreatment with capsaicin (75 mg/kg in total, sc). These results suggest that sympathetic nervous system, but not capsaicin-sensitive sensory nerves, be involved in the enhancements of GIT by **3**, **4**, and **6**. It is worthy to study their therapeutical effect in the prevention of the inhibition of GIT, including ileus, in clinic.

Introduction

There have been a few studies concerning the effects of saponins on gastrointestinal functions. Recently, as a continuing part of our studies of bioactive saponins, we found that oleanolic acid 3-*O*-monodesmosides showed the inhibitory effects on gastric emptying in rats and mice, and the protective effects on gastric lesions induced by ethanol or indomethacin in rats.[1–3] We have also reported that pretreatments with capsaicin and streptozotocin (STZ) markedly reduced the inhibition of momordin Ic on gastric emptying.[4] Gastric emptying and gastrointestinal transit (GIT) are very important functions of the digestive system. Ileus is the common complication induced by various reasons, such as laparotomy with manipulation, peritoneal irritation, etc. Precisely because we lack specific therapy, ileus remains an important clinical problem. Patients with ileus accumulate gas and secretions leading to bloating, distention, emesis, and pain. Prokinetic drugs, including cisapride, metoclopramide, erythromycin, and octreotide, are commonly used for chronic ileus. Unfortunately, in advanced cases, no medical therapy provides impressive relief.[5] Non-steroidal anti-inflammatory drugs, such as indomethacin, are known for their ability to block the prostaglandins biosynthesis and they are widely used for postoperative pain. These medicines are shown to be beneficial in the treatment of postoperative ileus in rodents.[6] However, they are also known to cause undesirable side effects.

In this report, we describe the effects of oleanolic acid (**1**), the 28-*O*-monodesmoside (compound O (**2**)), the 3-*O*-monodesmosides (oleanolic acid 3-*O*-glucuronide (**3**), momordins Ic (**4**) and I (**6**), and 28-deglucosyl-chikusetsusaponins IV (**8**) and V (**10**)), and the 3,28-*O*-bisdesmosides (momordin IIc (**5**) and chikusetsusaponins IV (**7**) and V (**9**)) on GIT and ileus induced by the peritoneal irritation or laparotomy with manipulation in fasted mice. We also discuss the effects of the active saponins on GIT in the capsaicin- or STZ-pretreated mice.

Results and Discussion

The effects of oleanolic acid (**1**) and its glycosides (**2**–**10**) on GIT and ileus in mice are summarized in Table 1.

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Table 1. Effects of oleanolic acid (**1**) and its glycosides (**2**–**10**) on GIT in mice

Treatment	Dose (mg/kg, po)	N	GIT (%)		
			Normal	Ileus by AcOH	Ileus by operation
Control	–	10	49.6 ± 2.8	12.9 ± 1.8	23.7 ± 1.7
Oleanolic acid (**1**)	50	8	50.2 ± 5.0	10.5 ± 1.7	22.1 ± 1.8
Compound O (**2**)	50	8	37.3 ± 1.5*	8.2 ± 0.9*	20.6 ± 1.6
Momordin IIc (**5**)	50	8	45.8 ± 2.7	10.4 ± 1.0	20.1 ± 1.2
Chikusetsusaponin IV (**7**)	50	8	42.9 ± 2.4	10.5 ± 0.8	25.6 ± 2.5
Chikusetsusaponin V (**9**)	50	8	51.8 ± 4.3	7.7 ± 0.6*	20.0 ± 2.1
Control	–	10	48.6 ± 2.5	14.1 ± 1.4	23.4 ± 1.8
Oleanolic acid 3-*O*-glucuronide (**3**)	12.5	8	43.4 ± 4.7	15.7 ± 1.2	28.0 ± 2.3
	25	8	49.5 ± 2.7	15.0 ± 0.8	26.5 ± 2.6
	50	8	69.3 ± 6.7**	21.2 ± 0.7	37.1 ± 4.4**
Momordin Ic (**4**)	2.5	8	–	14.8 ± 1.9	–
	5	8	–	29.5 ± 3.3**	–
	12.5	8	55.3 ± 2.2	36.1 ± 1.4**	23.8 ± 2.0
	25	8	65.3 ± 2.1**	48.9 ± 4.5**	25.8 ± 1.5
	50	8	70.2 ± 2.7**	–	38.8 ± 2.2**
Control	–	10	48.8 ± 2.3	13.1 ± 1.4	23.4 ± 1.4
Momordin I (**6**)	5	8	52.5 ± 2.2	19.6 ± 2.3	–
	12.5	8	60.7 ± 3.7	24.6 ± 2.2**	26.2 ± 1.8
	25	8	67.0 ± 6.2**	21.4 ± 2.4*	25.7 ± 2.8
	50	8	–	–	36.3 ± 2.3**
Control	–	10	47.8 ± 2.6	13.4 ± 1.5	22.6 ± 2.0
28-Deglucosyl-chikusetsusaponin IV (**8**)	25	8	59.8 ± 3.5	15.0 ± 1.3	24.7 ± 2.9
	50	8	42.8 ± 8.4	10.4 ± 1.5	24.6 ± 3.5
28-Deglucosyl-chikusetsusaponin V (**10**)	25	8	52.4 ± 2.2	12.9 ± 1.0	24.0 ± 1.8
	50	8	51.8 ± 4.3	15.7 ± 3.5	40.5 ± 2.3**
Control	–	10	47.2 ± 3.1	14.0 ± 0.8	21.8 ± 2.2
Metoclopramide	12.5	8	54.0 ± 2.5	13.6 ± 1.5	22.8 ± 2.1
	25	8	58.5 ± 2.7*	14.3 ± 1.3	24.4 ± 2.8
	50	8	59.2 ± 2.7**	21.4 ± 1.9*	33.4 ± 1.8**
Indomethacin	5	8	42.2 ± 2.4	25.5 ± 2.5**	35.0 ± 2.0**
	12.5	8	40.6 ± 3.0	23.4 ± 2.8**	36.9 ± 2.2**
	25	8	38.2 ± 3.6	32.1 ± 2.3**	38.4 ± 1.6**

Values represent the means ± SEM. Significantly different from the control group, *$p < 0.05$, **$p < 0.01$.

The present results demonstrated that, 1 h after the oral administration, three of the 3-*O*-monodesmosides (oleanolic acid 3-*O*-glucuronide (**3**, 50 mg/kg), momordin Ic (**4**, 25 and 50 mg/kg), momordin I (**6**, 25 mg/kg)) significantly accelerated GIT in normal mice, but two of the 3-*O*-monodesmosides (28-deglucosyl-chikusetsusaponin IV (**8**) and V (**10**)), the 3,28-*O*-bisdesmosides (momordin IIc (**5**), chikusetsusaponins IV (**7**) and V (**9**)), and the aglycon (Oleanolic acid (**1**)) (50 mg/kg) showed no significant effect. Whereas the 28-*O*-monodesmoside (compound O (**2**, 50 mg/kg)) significantly inhibited GIT. A reference drug, metoclopramide, slightly accelerated GIT, whereas indomethacin (5–25 mg/kg) tended to reduce GIT. Momordin Ic (**4**, 5–25 mg/kg), momordin I (**6**, 12.5 and 25 mg/kg), as well as reference drugs (metoclopramide (50 mg/kg) and indomethacin (5–25 mg/kg)), significantly prevented the inhibition of GIT induced by the peritoneal injection of acetic acid. Oleanolic acid 3-*O*-glucuronide (**3**) tended to prevent the inhibition of GIT. Compound O (**2**) and chikusetsusaponin V (**9**) (50 mg/kg) significantly potentiated the inhibition of GIT, while oleanolic acid (**1**), momordin IIc (**5**), chikusetsusaponin IV (**7**), and 28-deglucosyl-chikusetsusaponins IV (**8**) and V (**10**) showed no such effect at a dose of 50 mg/kg. Ninety-five minutes after the oral administration, four of the 3-*O*-monodesmosides (oleanolic acid 3-*O*-glucuronide (**3**), momordin Ic (**4**), momordin I (**6**), and 28-deglucosyl-chikusetsusaponins V (**10**)) (50 mg/kg), as well as reference drugs (metoclopramide (50 mg/kg) and indomethacin (5–25 mg/kg)), significantly prevented the inhibition of GIT induced by laparotomy with manipulation. Oleanolic acid (**1**), compound O (**2**), momordin IIc (**5**), chikusetsusaponins IV (**7**) and V (**9**), and 28-deglucosyl-chikusetsusaponins IV (**8**) (50 mg/kg) showed no significant effect.

In this study, oleanolic acid (**1**) and the 28-ester glucosides (**2**, **5**, **7**, **9**) did not accelerate GIT in any models. On the other hand, several of the 3-*O*-monodesmoside (**3**, **4**, **6**) accelerated GIT in normal mice, and prevented the inhibition of GIT induced by peritoneal irritation or laparotomy with manipulation (**10** showed pereventive activity only in this experiment). These results indicate that the 3-*O*-glycoside moiety is essential to show the activity and the 28-ester glucoside moiety reduces the activity.

Capsaicin is widely used to ablate sensory C fibers. It has been systematically used to ablate all capsaicin-sensitive C fibers.[7] Hyperglycemia in STZ-induced hypoinsulinemic rats reduced the sensitivity of the sympathetic nervous system.[8] As shown in Table 2, the accelerations of GIT by oleanolic acid 3-*O*-glucuronide (**3**) and momordins Ic (**4**) and I (**6**) were completely abolished

Table 2. Effects of oleanolic acid 3-*O*-glucuronide (**3**) and momordins Ic (**4**) and I (**6**) on GIT in capsaicin- or STZ-pretreated mice

Treatment	Dose (mg/kg, po)	N	GIT (%)	
			Capsaicin-pretreated mice	STZ-pretreated mice
Normal	–	9	49.4 ± 1.6	47.7 ± 2.8
Control	–	10	46.2 ± 1.7	47.6 ± 1.6
Oleanolic acid 3-*O*-glucuronide (**3**)	50	8	64.5 ± 2.5**	50.6 ± 3.4
Momordin Ic (**4**)	25	8	61.4 ± 3.4**	46.6 ± 2.6
	50	8	67.7 ± 4.3**	47.6 ± 2.7
Momordin I (**6**)	25	8	65.5 ± 4.4**	54.3 ± 2.8

Values represent the means ± SEM. Significantly different from the control group, $^{**}p < 0.01$.

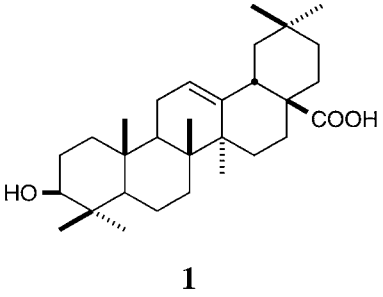
by the pretreatment with STZ (100 mg/kg, iv), but not with capsaicin (75 mg/kg in total, sc). These results suggest that sympathetic nervous system, but not capsaicin-sensitive sensory nerves, seem to be involved in the enhancements of GIT by **3**, **4**, and **6**.

We have reported that oleanolic acid 3-*O*-monodesmosides (**3**, **4**, **6**, **8**, **10**) inhibited gastric emptying in mice.[2] It is interesting that some of them accelerated GIT in normal mice and/or prevented the inhibition of GIT induced by acetic acid or laparotomy with manipulation. No drug having both the gastric emptying inhibitory activity and the GIT accelerating activity is known. For example, metoclopramide accelerates both gastric emptying and GIT,[9] and reversely, clonidine inhibits both of them.[10] It is worthy to study their mechanisms and therapeutical effect in the prevention and treatment of the inhibition of GIT, including ileus, in clinic.

Experimental

Materials

Oleanolic acid 3-*O*-glucuronide (**3**) and momordins Ic (**4**), IIc (**5**), and I (**6**) were isolated from the fruit of *Kochia scoparia* (L.) Schrad.,[11] or the seeds of *Momordica cochinchinesis* Spreng.[12] Chikusetsusaponins IV (**7**) and V (**9**) were isolated from the rhizome of *Panax japonicus* C. A. Meyer, and 28-deglucosyl-chikusetsusaponins IV (**8**) and V (**10**) were obtained by alkaline hydrolysis of **7** and **9**, respectively.[13]



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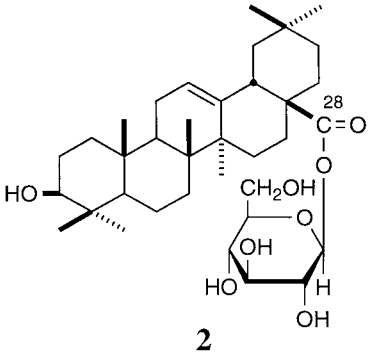

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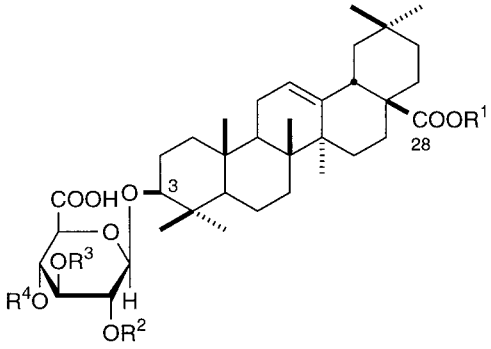

	R^1	R^2	R^3	R^4
3 :	H	H	H	H
4 :	H	H	Xyl	H
5 :	Glc	H	Xyl	H
6 :	H	H	Ara(p)	H
7 :	Glc	H	H	Ara(f)
8 :	H	H	H	Ara(f)
9 :	Glc	Glc	H	H
10 :	H	Glc	H	H

Glc : β-D-glucopyranosyl
Xyl : β-D-xylopyranosyl
Ara(p) : α-L-arabinopyranosyl
Ara(f) : α-L-arabinofuranosyl

Figure 1. Chemical structures of oleanolic acid (**1**) and its glycosides (**2**–**9**).

Compound O (**2**) was obtained by enzymatic hydrolysis of **9**.[1] Other reagents were purchased from Wako Pure Chemical Industries, Japan.

Animals

Male ddY mice, weighing 27–30 g, were purchased from Kiwa Laboratory Animal Co., Ltd., Japan. The animals were maintained at a constant temperature of 23 ± 2 °C and were fed a standard laboratory chow (MF, Oriental Yeast Co., Ltd., Japan) for a week. The animals were fasted for 18–20 h prior to experiments, but were supplied with water ad libitum. Each test sample was suspended in 5% acacia/phosphate buffered saline solution, and the solution was orally administered at 10 mL/kg in each experiment, while the vehicle was administered orally at 10 mL/kg in the corresponding control group.

Measurement of GIT in normal mice

A charcoal meal containing a solution of 1.5% carboxymethyl cellulose sodium salt (CMC-Na) and 5% charcoal as a marker was intragastrically given (0.2 mL/mouse) to conscious mice. Thirty minutes later, mice were sacrificed by cervical dislocation. The abdominal cavity was opened, and the gastrointestinal tract was removed. The traveled distance of the marker was measured and expressed as a percentage of the total length of the small intestine from pylorus to caecum. The test samples were orally given by means of a metal orogastric tube 60 min prior to the administration of the charcoal meal. In pre-experiment, momordin I (**6**) at a dose of 50 mg/kg showed scattering values (51.3 ± 12.2, N = 8), which seemed to be due to its potent inhibitory activity of gastric emptying. Therefore, 25 mg/kg was chosen as maximum dose of **6** in this model.

Measurement of GIT in peritoneal-irritated mice

The peritoneal-irritation ileus was induced by a modification of the method described by Riviere et al.[14] Acetic acid saline solution (1%, 0.2 mL/mouse) was peritoneally injected 30 min after the oral administration of the samples. The charcoal meal was administered 30 min after the injection of acetic acid. GIT was determined as described above. Momordins Ic (**4**) and I (**6**) at a dose of 50 mg/kg also showed scattering values (36.2 ± 15.4 (N = 8), 19.2 ± 6.0 (N = 8)) in pre-experiment. Therefore, 25 mg/kg was chosen as maximum dose of **4** and **6** in this model.

Measurement of GIT in laparotomized and manipulated mice

The postoperative ileus was induced as the method described by De Winter et al.[15] Briefly, the mice underwent an operation under ether anesthesia. In contrast with pentobarbital anesthesia, ether anesthesia was chosen, whose effect on gastrointestinal motility approximately lasted for only 1 h after the induction of the anesthesia.[16] The abdominal cavity was opened, and then the small intestine and caecum were gently pulled out of the abdominal cavity and unfurled like a fan on two sterile gauzes covering the abdomen of the mouse. After 5 min of gentle manipulation, the small intestine and caecum were replaced in the abdominal cavity and the surgical wound was sutured. After the operation, the mice were allowed to recover for 60 min. Test samples were orally given 30 min before the operation. The charcoal meal was administered 65 min after the operation. GIT was determined as described above.

Measurement of GIT in STZ-induced hyperglycemic mice

STZ (100 mg/kg, dissolved in 10 mL citrate buffer (pH 4.2), iv) was administered to the 20-h fasted mice. Four weeks later, blood samples were collected from the retro-orbital sinus under the unfasted condition. Serum glucose levels were determined by the glucose-oxidase method (kit reagent: Glucose CII-test Wako, Wako Pure Chemical Industries). Mice with a serum glucose level above 600 mg/dL, considered to be diabetic, were used in this study. The charcoal meal was administered 60 min after the oral administration of the samples. GIT was determined as described above.

Measurement of GIT in capsaicin-pretreated mice

Capsaicin solution was prepared in a solution containing 99.5% ethanol, Tween 80, and saline (2:1:7, v/v/v). Mice were anesthetized with sodium pentobarbital (30 mg/kg, ip), and treated with increasing doses of capsaicin for two consecutive days (25 and 50 mg/kg, sc) to deplete neuropeptides in primary afferent neurons as a modification of the method described previously.[7] To counteract any respiratory impairment associated with administration of capsaicin, the mice were pretreated with aminophylline (10 mg/kg, dissolved in 5 mL saline, im) 30 min before capsaicin injection. After 14 days, the efficiency of capsaicin pretreatment was verified by the corneal chemosensory test which consists of monitoring the wiping reflex to ocular instillation of a drop of 0.1% NH_4OH solution. None of the capsaicin-pretreated mice showed a wiping response, indicating effective ablation of primary sensory afferents, whereas wiping reflex was present in vehicle-pretreated mice. The charcoal meal was administered 60 min after the oral administration of the samples. GIT was determined as described above.

Statistics

Values were expressed as means ± SEM. One-way analysis of variance following Dunnett's test for multiple comparison analysis was used for statistical analysis. Probability (p) values less than 0.05 were considered significant.

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