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STRUCTURE-RELATED ENHANCING ACTIVITY OF ESCINS Ia, Ib, IIa AND IIb ON MAGNESIUM ABSORPTION IN MICE

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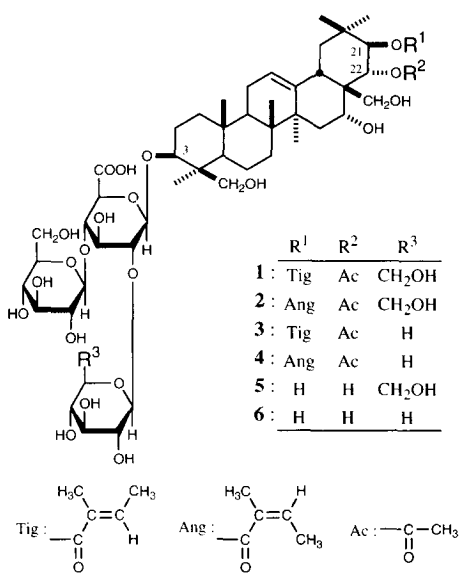
Abstract: We examined the effects of the saponin fraction and its principal saponins, escins Ia (**1**), Ib (**2**), IIa (**3**) and IIb (**4**), obtained from European horse chestnut, and their hydrolyzed products, desacylescins I (**5**) and II (**6**) on magnesium absorption from the gastrointestinal tract in mice. Test samples were given orally to fasted mice before loading of 0.5 or 1.67 M $MgSO_4$ (10 mL/kg, *p.o.*). The saponin fraction (12.5–100 mg/kg) significantly enhanced the Mg^{2+} absorption 30, 60, 120 and 240 min after administration, with maximum enhancement by 48.3% at 50 mg/kg. Escins Ib (**2**) and IIb (**4**) (12.5 and 25 mg/kg) also enhanced the absorption, whereas escins Ia (**1**) and IIa (**3**) (12.5 and 25 mg/kg) and desacylescins I (**5**) and II (**6**) (25 mg/kg) showed no activity. These results suggested that the 21-*O*-tigloyl and/or 22-*O*-acetyl group(s) is essential for such activity. The saponin fraction, **2** and **4** (50 mg/kg) also affected the activity, but their effects were attenuated in streptozotocin-induced diabetic mice. Furthermore, pretreatment with insulin or indomethacin did not reduce the effect of **2** and **4**. These results also implied that neither the sympathetic nervous system nor endogenous prostaglandins were involved. The involvement of parathyroid hormone, and/or the metabolism of vitamin D should be considered.

Magnesium is an intracellular cation. It is an essential element which catalyzes more than 300 enzymatic reactions, in particular those involving ATP. Despite the ubiquitous nature of Mg, low serum Mg^{2+} occurs either due to decreased absorption or increased excretion. Hypomagnesemia is surprisingly common in hospital populations. Mg deficiency may result in hypokalemia and hypocalcemia. Myocardial Mg depletion may result in influx of Na^+ and Ca^{2+} into the mitochondria, which may in turn lead to myocardial cell death. Hence, low Mg^{2+} concentration may be a factor for a wide variety of clinical conditions[1]. Diabetes mellitus is the most frequent chronic disease associated with secondary Mg deficit. Hypomagnesemia is a central feature of the deficit, which is often reported in experimental and clinical forms of the disease. In diabetic rats, as in man, plasma Mg^{2+} concentrations may be correlated inversely with the degree of hyperglycemia. The duration of the disease also appears to be relevant. Clinical studies have speculated on a potential link between the Mg deficit in diabetes and several diabetic complications including cardiovascular problems and retinopathy. Myocardial



	R^1	R^2	R^3
1 :	Tig	Ac	CH_2OH
2 :	Ang	Ac	CH_2OH
3 :	Tig	Ac	H
4 :	Ang	Ac	H
5 :	H	H	CH_2OH
6 :	H	H	H

Figure 1. Chemical structures of escins Ia (**1**), Ib (**2**), IIa (**3**) and IIb (**4**) and desacylescins I (**5**) and II (**6**)

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disorders associated with Mg deficiency have been reported in diabetic mice and rabbits. It is possible that a common mechanism involving Mg may be responsible for some of the diverse complications of diabetes. The etiology of hypomagnesemia in diabetes is complex. Nevertheless, plasma Mg^{2+} concentrations are ultimately determined by four processes: intake, gastrointestinal absorption, redistribution within body pools, and urinary excretion.[2]

We have reported the inhibitory activities of saponin fraction and its principal saponins, escins Ia (**1**), Ib (**2**), IIa (**3**) and IIb (**4**) isolated from the seeds of *Aesculus hippocastanum*, on the increase of blood glucose or ethanol concentration in oral glucose- or ethanol-loaded rats,[3] inhibition of glucose uptake in the small intestine *in vitro*, lowering of gastric emptying in rats and mice,[4,5] acceleration of gastrointestinal transit in mice,[6] anti-inflammatory effects in rats and mice,[7,8] and gastroprotection in rats.[9] In this study, we investigated the effects of the saponin fraction, its principal saponins, escins Ia (**1**), Ib (**2**), IIa (**3**) and IIb (**4**), and their hydrolyzed products, desacylescins I (**5**) and II (**6**), on Mg^{2+} absorption from the gastrointestinal tract in mice. The possible mechanisms were also discussed.

Results and Discussion

The effects of the saponin fraction, escins Ia–IIb (**1**–**4**) and desacylescins I (**5**) and II (**6**) on the serum Mg^{2+} levels in 0.5 or 1.67 M $MgSO_4$ (10 mL/kg)-loaded mice are summarized in Table 1. The present results

Table 1. Effects of the saponin fraction on Mg^{2+} absorption in $MgSO_4$-loaded mice

Treatment	Dose (mg/kg, *p.o.*)	N	Serum Mg^{2+}	Increase (%)
Normal	-	8	2.9±0.1**	-
1.67 M $MgSO_4$-loaded (10 mL/kg, *p.o.*)				
Control	-	12	5.8±0.2	-
Saponin Fraction	2.5	10	5.6±0.4	-3.4
	5	10	5.9±0.3	1.7
	12.5	10	8.1±0.3**	39.7
	25	10	7.8±0.4**	34.5
	50	10	8.6±0.6**	48.3
	100	10	7.4±0.4**	27.6
0.5 M $MgSO_4$-loaded (10 mL/kg, *p.o.*)				
Control	-	8	3.3±0.1	-
Saponin Fraction	5	8	3.2±0.1	-3.0
	12.5	8	3.8±0.1*	15.2
	25	8	4.6±0.2**	39.4
0.5 M $MgSO_4$-loaded (10 mL/kg, *p.o.*)				
Administration 30 min before $MgSO_4$				
Control	-	8	3.2±0.1	-
Saponin Fraction	25	8	4.6±0.2**	43.8
Administration 120 min before $MgSO_4$				
Control	-	8	3.2±0.0	-
Saponin Fraction	25	8	3.8±0.1**	18.8
Administration 240 min before $MgSO_4$				
Control	-	8	3.2±0.1	-
Saponin Fraction	25	8	3.4±0.1*	6.3
Administration 300 min before $MgSO_4$				
Control	-	8	3.2±0.1	-
Saponin Fraction	25	8	3.2±0.1	0.0

Each value represents the mean±S.E.M. Significantly different from the control group. *$p<0.05$, **$p<0.01$.

demonstrated that the saponin fraction (12.5–100 mg/kg, *p.o.*) significantly increased serum Mg^{2+} levels with maximum enhancement by 48.3% at 50 mg/kg in 1.67 M $MgSO_4$-loaded mice, and by 39.4% in 0.5 M $MgSO_4$-loaded mice. The optimal time of the effect was 30–240 min after administration of the sample. The effect disappeared 300 min after administration. We have reported previously that the saponin fraction markedly inhibited gastric emptying in mice.[5] In the present study, the increased dosages (25–100 mg/kg) of the saponin fraction did not markedly increase the enhancement. This was probably attributable to the inhibitory activity of the saponin fraction on gastric emptying.

As shown in Table 2, escins Ib (**2**) and IIb (**4**) (12.5–50 mg/kg, *p.o.*) also increased the serum Mg^{2+} levels, whereas escins Ia (**1**) and IIa (**3**) (5–25 mg/kg, *p.o.*) and desacylescins I (**5**) and II (**6**) (25 mg/kg, *p.o.*) showed no significant effect, in 0.5 M $MgSO_4$-loaded mice. As shown in Table 3, the saponin fraction, **2** and **4** still increased the serum Mg^{2+} levels in streptozotocin-induced diabetic and 1.67 M $MgSO_4$-loaded mice. As shown in Table 4, the saponin fraction, **2** and **4** (25 mg/kg) did not increase the serum Mg^{2+} levels in $MgSO_4$-unloaded mice. These results suggested that the saponin fraction and escins Ib (**2**) and IIb (**4**) increase serum Mg^{2+} levels by enhancing Mg^{2+} absorption from the gastrointestinal tract in mice. We have reported previously that the saponin fraction and escins Ib (**2**) and IIb (**4**) inhibited the gastric emptying in normal and streptozotocin-induced diabetic mice, which may be of benefit to diabetic patients in control of postpradial glucose level.[5] The enhancement of Mg absorption from the gastrointestinal tract may be also of benefit to diabetic patients for improvement of hypomagnesemia.

Table 2. Effects of escins (**1**–**4**) and desacylescins (**5**, **6**) on Mg^{2+} absorption in $MgSO_4$-loaded mice

Treatment	Dose (mg/kg, *p.o.*)	N	Serum Mg^{2+}	Increase (%)
0.5 M $MgSO_4$-loaded (10 mL/kg, *p.o.*)				
Control	-	8	3.3±0.1	-
Escin Ia (**1**)	5	8	3.6±0.1	9.1
	12.5	8	3.3±0.1	0.0
	25	8	3.6±0.2	9.1
Escin Ib (**2**)	5	8	3.7±0.2	12.1
	12.5	8	3.9±0.1*	18.2
	25	8	4.1±0.2**	24.2
Desacylescin I (**5**)	25	8	3.3±0.2	0.0
Desacylescin II (**6**)	25	8	3.4±0.1	3.0
0.5 M $MgSO_4$-loaded (10 mL/kg, *p.o.*)				
Control	-	10	3.2±0.1	-
Escin IIa (**3**)	5	8	3.0±0.1	-6.3
	12.5	8	3.1±0.2	-3.1
	25	8	3.6±0.2	12.5
Escin IIb (**4**)	5	8	3.2±0.0	0.0
	12.5	8	4.0±0.2**	25.0
	25	8	4.0±0.2**	25.0

Each value represents the mean±S.E.M. Significantly different from the control group, *$p<0.05$, **$p<0.01$.

In the present study, desacylescins I (**5**) and II (**6**), lacking the acyl groups at 21 and 22 positions, did not show the activity. Furthermore, escins Ib (**2**) and IIb (**4**) with an angeloyl group, but not escins Ia (**1**) and IIa (**4**) with a tigloyl group, were also found to show significant effects. These results suggested that the 21-*O*-tigloyl and/or 22-*O*-acetyl group(s) is essential for the activity.

Indomethacin is an inhibitor of prostaglandin biosynthesis. We reported previously that the effects of escins (**1**–**4**) on ethanol-induced gastric mucosal lesions were markedly attenuated by pretreatment with indomethacin, which suggested that endogenous prostaglandins were commonly involved in the effects.[9] In the present study, pretreatment with indomethacin did not reduce the effect of escins Ib (**2**) or IIb (**4**) on Mg^{2+} absorption (Table 3). These results suggested that endogenous prostaglandins is not involved in the effects of escins (**2**, **4**) on Mg^{2+} absorption in mice.

It has been reported that insulin induced arousal of the sympathetic nervous system.[10] The activity of the sympathetic nervous system was shown to be abnormal in diabetic animals.[11] Parathyroid hormone has been shown to increase Mg absorption,[12] possibly in part by promoting metabolism of vitamin D to active products such as 1,25-dihydroxycholecalciferol.[13,14] Vitamin D also promotes absorption of Mg.[15–17] Mg absorption from the small intestine was depressed in diabetic rats.[18] Both hyperparathyroidism and abnormal vitamin D metabolism probably occur in the diabetic rats, and thus hyperparathyroidism would be expected to increase and abnormal vitamin D metabolism to decrease duodenal absorption of Mg, calcium and strontium.[18] Since the parathyroid hormone probably acts on transport through effects on vitamin D metabolism, the primary effect on transport in diabetes should be a consequence of abnormalities in vitamin D metabolism. Hence, depression of absorption is to be expected for both two elements.

Table 3. Effects of saponin fraction and escins (**2**, **4**) on Mg^{2+} absorption in $MgSO_4$-loaded mice

Treatment	Dose (mg/kg, *p.o.*)	N	Serum Mg^{2+}	Increase (%)
1.67 M $MgSO_4$-loaded (10 mL/kg, *p.o.*)				
Normal (STZ-untreated)				
Control	-	8	5.7±0.3	-
Saponin Fraction	50	8	8.4±0.4**	47.4
Escin Ib (**2**)	50	8	7.9±0.4**	38.6
Escin IIb (**4**)	50	8	8.0±0.5**	40.4
$MgSO_4$-unloaded				
Normal (STZ-untreated)	-	10	2.9±0.1	-
STZ-treated	-	15	2.1±0.1**	-
1.67 M $MgSO_4$-loaded (10 mL/kg, *p.o.*)				
STZ (100 mg/kg, i.v.)-pretreated				
Control	-	11	5.0±0.2	-
Saponin Fraction	50	11	6.4±0.5*	28.0
Escin Ib (**2**)	50	10	6.3±0.3*	26.0
Escin IIb (**4**)	50	10	6.3±0.4*	26.0
0.5 M $MgSO_4$-loaded (10 mL/kg, *p.o.*)				
Insulin (1 U/kg, s.c.)-pretreated				
Control	-	10	3.9±0.1	-
Escin Ib (**2**)	12.5	10	5.1±0.3**	30.8
Escin IIb (**4**)	12.5	10	5.0±0.2**	28.2
0.5 M $MgSO_4$-loaded (10 mL/kg, *p.o.*)				
Indomethacin (10 mg/kg, s.c.)-pretreated				
Control	-	10	3.2±0.1	-
Escin Ib (**2**)	12.5	10	4.3±0.2**	34.4
Escin IIb (**4**)	12.5	10	4.1±0.2**	28.1

Each value represents the mean±S.E.M. Significantly different from the control group, *$p<0.05$, **$p<0.01$.

Table 4. Effects of the saponin fraction and escins (**1**–**4**) on serum Mg^{2+} in normal mice

Treatment	Dose (mg/kg, *p.o.*)	N	Serum Mg^{2+}	Increase (%)
$MgSO_4$-unloaded				
Control	-	12	3.0±0.1	-
Saponin Fraction	25	10	2.9±0.2	-3.3
Escin Ia (**1**)	25	8	3.1±0.1	3.3
Escin Ib (**2**)	25	8	3.1±0.1	3.3
Escin IIa (**3**)	25	8	3.1±0.2	3.3
Escin IIb (**4**)	25	8	3.1±0.2	3.3

Each value represents the mean±S.E.M. No significant differences were observed with respect to the control group.

As shown in Table 3, the effects of the saponin fraction and escins Ib (**2**) and IIb (**4**) were attenuated in streptozotocin-induced diabetic mice, but not in insulin-pretreated mice. These results excluded the involvement of the sympathetic nervous system in the effects of escins (**2**, **4**) on Mg^{2+} absorption from the gastrointestinal tract in mice. The effects of parathyroid hormone and/or metabolism of vitamin D should be considered.

Experimental

Materials

Saponin fraction and escins Ia (**1**), Ib (**2**), IIa (**3**) and IIb (**4**) were isolated from the seeds of *Aesculus hippocastanum* using our method reported previously.[3] Other reagents were purchased from Wako Pure Chemical Industries, Japan.

Bioassay Methods

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 standard laboratory chow (MF, Oriental Yeast Co., Ltd., Japan) for one week. The animals were fasted for 18–20 h prior to experiments, but were given water *ad libitum*. Each test sample was dissolved in phosphate buffered saline solution, and the solution was administered orally at 10 mL/kg in each experiment, while the vehicle was administered orally at 10 mL/kg in the corresponding control group.

Measurements of Serum Mg^{2+} and Glucose: Blood samples were collected from the retro-orbital sinus in order to measure serum Mg^{2+} or glucose levels. Serum Mg^{2+} levels were determined by the xylidyl blue method (kit reagent: Magnesium B-test Wako, Wako Pure Chemical Industries). Serum glucose levels were determined by the glucose-oxidase method (kit reagent: Glucose CII-test Wako, Wako Pure Chemical Industries).

Absorption of Mg^{2+} from the Gastrointestinal Tract in $MgSO_4$-loaded Mice: The fasted mice were placed in separate cages for 2 h. The test samples were given orally by means of a metal orogastric tube, and 0.5 or 1.67 M $MgSO_4$ solution (10 mL/kg) was administered orally 30, 120, 180, 240 or 300 min later. Serum Mg^{2+} levels were determined 60 min after administration of the $MgSO_4$ solution.

Absorption of Mg^{2+} from the Gastrointestinal Tract in Streptozotocin-induced Diabetic and $MgSO_4$-loaded Mice: Streptozotocin (STZ, 100 mg/kg, *i.v.*), dissolved in 10 mL citrate buffer (pH 4.2), was administered to the 20-h fasted mice 4 weeks before administration of the samples. Mice with a serum glucose level above 600 mg/dL under unfasted conditions, considered to be diabetic, were used in this study. The 1.67 M $MgSO_4$

solution (10 mL/kg) was administered orally 30 min after the samples. Serum Mg^{2+} levels were determined 60 min after administration of the $MgSO_4$ solution.

Absorption of Mg^{2+} from the Gastrointestinal Tract in Indomethacin-pretreated and $MgSO_4$-loaded Mice: Indomethacin (10 mg/kg, dissolved in 5% $NaHCO_3$, and diluted in distilled water, *s.c.*) was administered to the 20-h fasted mice 30 min before administration of the samples. The 0.5 M $MgSO_4$ solution (10 mL/kg) was administered orally 30 min after the samples. Serum Mg^{2+} levels were determined 60 min after administration of the $MgSO_4$ solution.

Absorption of Mg^{2+} from the Gastrointestinal Tract in Insulin-pretreated and $MgSO_4$-loaded Mice: Insulin (1 U/kg, dissolved in diluted HCl, and diluted in distilled water, *s.c.*) was administered to the 20-h fasted mice 30 min before administration of the samples. The 0.5 M $MgSO_4$ solution (10 mL/kg) was administered orally 30 min after the samples. Serum Mg^{2+} levels were determined 60 min after administration of the $MgSO_4$ solution.

Serum Mg^{2+} levels in Normal Mice: The fasted mice were placed in separated cages for 2 h. Test samples were given orally, and serum Mg^{2+} levels were determined 90 min after administration of the samples.

Statistics: Values are expressed as means±S.E.M. For statistical analysis, Student's t-test or one-way analysis of variance following Dunnett's test for parametric data was used. Probability (p) values less than 0.05 were considered significant.

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