ENHANCED SMALL INTESTINAL ABSORPTION OF β-LACTAM ANTIBIOTICS IN RATS IN THE PRESENCE OF MONODESMOSIDES ISOLATED FROM PERICARPS OF SAPINDUS MUKUROSSI (ENMEI-HI)\(^1,2\)

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Monodesmoside, saponin A, B and C, isolated from pericarps of Sapindus mukurossi (Enmei-hi) have been shown to promote absorption of poorly absorbed β-lactam antibiotics by the small intestine using an in situ loop method.

Monodesmosides were solubilized with ginseng crude saponin extract, a mixture of bisdesmosides, saponin X, Y\(\text{I}\) and Y\(\text{II}\) which were isolated from pericarps of Sapindus mukurossi and/or a sesquiterpene-oligoglycoside isolated also from Sapindus mukurossi. These solubilizing agents were demonstrated not to influence the absorption promoting effect of monodesmosides. Among the monodesmosides, saponin B showed the greatest effect. No influence of osmolarity of the administered solution on the absorption promoting action was observed. The promoting functions of the three monodesmosides for the small intestinal absorption of antibiotics were suppressed by Ca\(^{2+}\) ion coexisting in the administered solution.

**Keywords** — enhanced intestinal absorption; β-lactam antibiotics; ampicillin; cephalothin; cefazolin; absorption promoter; monodesmoside; bisdesmoside; solubilizing agent

Clinically, there is great interest in improving gastro-intestinal absorption of drugs. This interest is demonstrated by the many studies that have been reported\(^2–6\) on the promoting effects of adjuvants for rectal absorption of poorly absorbed drugs. In addition, there are many reports on the promotion of small intestinal absorption of poorly absorbed drugs such as phenol red,\(^7,8\) sulfaguanidine,\(^9\) heparin\(^10,11\) and antibacterial antibiotics.\(^12,14\) However, there are few reports on the promoters of absorption isolated from traditional crude drugs. In the previous paper, we reported the enhancing effect of three monodesmosides, saponin A, B and C, on the rectal absorption of β-lactam antibiotics in rats.

In the present paper, we report that monodesmosides also facilitate the small intestinal absorption of normally poorly absorbed β-lactam antibiotics in rats and that the calcium ion coexisting with the monodesmosides suppresses the absorption enhancing effect.

**EXPERIMENTAL**

**Materials** — Sodium ampicillin (ABPC), sodium cephalothin (CEP) sodium cephalothin (CET) and sodium cefazolin (CEZ) were purchased from commercial sources and were used without further purification.

Monodesmosides: Saponin A, B and C (Chart 1), the purity of which were confirmed by thin-layer chromatography (TLC), carbon-13 nuclear magnetic resonance (\(^{13}\)C-NMR) and elemental analysis, were used.

Bisdesmosides: A mixture of mukurozi saponin X, Y\(\text{I}\) and Y\(\text{II}\) (XY-mix) (Chart 1), each of which was isolated from pericarps of Sapindus mukurossi by Kimata \(et\ al.,\)^1\(^5\) was used as a solubilizing agent for monodesmosides. Because of insufficient amounts of each saponin in Sapindus mukurossi, a mixture of 1:7:7 for X, Y\(\text{I}\) and Y\(\text{II}\), respectively, was used.

Ginseng Crude Saponin Extract: Ginseng was extracted with hot methanol followed by evaporation of the solvent. The extracts were suspended in water and defatted with ethyl ether. The

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defatted aqueous layer was extracted with n-
BuOH (saturated with water) followed by
evaporation of the solvent. After being deisic-
eted in vacuum at 40 °C for 2 d, the residues
were used as ginseng crude saponin extracts (G-mix).
G-mix was used as an solubilizing agent
for monodesmosides at a concentration of 3% in a
buffer solution.

Sesqui-terpene-Diglycosides: Four sesqui-
-terpene-diglycosides were also isolated from
pericarps of *Sapindus mukurossi* by Kuzuki et
al.16) Their chemical structures were identified
as shown in Chart 2. These compounds were
found to increase the solubility of the studied
monodesmosides.15) Among these compounds,
I1b was used as a solubilizing agent for monodes-
mosides in the present study.

**Methods** — (1) Intestinal Loop Experiment:
Male Wistar rats (200—230 g) were fasted for
16 h prior to experiments but water was given
freely. They were anesthetized by intraperitoneal
injection of sodium pentobarbital at a dose of 30
mg/kg and held supine on a surface kept at 37 °C
to maintain their body temperature above 36 °C.

The small intestines were exposed by an ab-
dominal incision and the bile duct was ligated. A
polyethylene tubing (PE 50) was inserted in the
distal direction at the duodenum and tied firmly
to keep it in position. A distal small intestine was
tied and the resulting intestinal loop of 10 cm
was exposed to a drug solution which was intro-
duced through the polyethylene tubing. Since
saponin A, B and C were sparingly soluble in
water, each monodesmoside was dissolved in pH
6.5 phosphate buffer with an aid of XY-mix
(0.2%), I1b (0.2%) or G-mix (3%). After dissolv-
ing an antibiotic in a solution containing a
monodesmoside solubilized with one of solu-
bilizing agents, the osmolality of the solution
was adjusted to 435 mOsmol/kg H₂O with
NaCl. A dose of an antibiotic and the volume of
drug solution administered were 50 mg/2.5
ml/kg. Blood samples were taken from a jugular
vein with a heparinized syringe at appropriate
time intervals and were used for the assay of the
antibiotics.

(2) Effect of Ca²⁺ Ions on the Absorption
Promoting Efficacy of Saponin A, B and C: In
order to examine the effect of the calcium ion on
the action of monodesmosides as an absorption
promoter, calcium chloride was added to the so-
lution introduced into the intestinal loop. A
1/150 M Triethylammonium-HCl buffer (pH 6.5)
was used instead of phosphate buffer.

Analytical Methods: The concentration of an-
tibiotics in whole blood (ABPC) or plasma
(other antibiotics) was determined by microbi-
oassay with *Bacillus subtilis* ATCC 6633 as the
test organism. Plasma or whole blood samples
were diluted with water more than 6-fold to
minimize the effect of plasma protein binding
on antibacterial activity of the antibiotics. Assay

![Chart 1](chart1.png)

**Chart 1.** Chemical Structures of Mono- and Bisdesmosides Isolated from Percarps of *Sapindus muku-
rossi* GAERTN.
Enhanced Intestinal Absorption

![Chemical Structures of Sesquiterpene-Oligoglycosides Isolated from Percarps of Sapindus mukurossi GAERTN.](image)

**CHART 2**

limits in plasma and whole blood were 0.075 µg/ml for ABPC, 0.5 µg/ml for CET 0.5 µg/ml for CEP and 0.75 µg/ml for CEZ.

Determination of Calcium Ion Sequestration Capacity: Calcium ion sequestration capacities of monodesmoside were determined by the dye indicator method described in a previous paper.60

RESULTS AND DISCUSSION

Enhanced Small Intestinal Absorption of β-Lactam Antibiotics by Monodesmosides in in Situ Loop Experiments

In order to assess the effectiveness of saponin A, B and C as an adjuvant to promote the absorption of poorly absorbed β-lactam antibiotics from small intestines, concentrations of the antibiotic in blood or plasma were determined after administration of an antibiotic into the small intestinal loop together with various amounts of saponin A, B or C. Fig. 1 shows the concentrations of ABPC in blood after administration of ABPC with various amounts of saponin A solubilized with 3% G-mix in the solution. The concentration of ABPC in blood was increased with an increase in the concentration of saponin A up to a dose of 1.0 mg/kg (corresponding to a concentration of 0.04% in the solution).

Similar effects of saponin A on the intestinal absorption of ABPC were observed in the case where it was solubilized with YX-mix or IIb. The maximum concentration of ABPC in blood ($C_{\text{max}}$) and the area under the concentration-time curve for 120 min ($AUC_{0-120}$) in the presence of saponin A solubilized with each solubilizing agent are shown in Fig. 2.

All solubilizing agents had no effect on the absorption of ABPC. Therefore, in the following studies, the XY-mix was mainly used as a solubilizing agent for monodesmosides.

The effects of monodesmosides at a dose of 2 mg/kg on small intestinal absorption of ABPC are shown in Fig. 3 in terms of $C_{\text{max}}$ and $AUC_{0-120}$.

All monodesmosides markedly enhanced the intestinal absorption of ABPC. Saponin B showed the greatest enhancement.

The effect of saponin A on the small intestinal absorption of other poorly absorbed β-lactam antibiotics was also examined. As shown in Fig. 4, the absorption of all the antibiotics studied was markedly increased in the presence of saponin A. Other monodesmosides also gave similar results.

Factors Influencing on the Absorption Promoting Action of Monodesmosides

1. Effect of Osmotic Pressure on the Solution

![Graph showing blood concentration over time](image)
FIG. 2. Enhanced Intestinal Absorption of ABPC in the Presence of Saponin A Solubilized by Either G-Mix, XY-Mix or IIb

Dose of ABPC: 50 mg/kg. Vol. of administration: 2.5 ml/kg. Dose of saponin A: 2 mg/kg. Concentration of solubilizing agents: G-mix, 3.0%; XY-mix, 0.2%; IIb, 0.2%. Saponin A was solubilized by each of solubilizing agent. Control contains ABPC and a corresponding solubilizing agent. Each point represents mean ± S.E. of 4—6 rats. Significantly different from control, a) p<0.01.

FIG. 3. Comparison of the Effects of Monodesmosides on the Intestinal Absorption of ABPC

Dose: ABPC 50 mg/kg; saponin A, B and C 2 mg/kg. Vol. of administration: 2.5 ml/kg. Control contains ABPC and XY-mix. Each monodesmoside was solubilized by XY-mix. Each point represents the mean ± S.E. of 4—6 rats. Significantly different from control, a) p<0.01.
Administered — Kitazawa et al.\textsuperscript{17} reported that small intestinal absorption of drugs from hypertonic perfusate was superior to that from isotonic solution due to the increase in water inflow. On the other hand, studies in our laboratory showed that the rectal absorption promoting efficacy of \( N \)-acylcollagen peptide was greater in hypertonic solution than in isotonic or hypertonic solution.\textsuperscript{4} However, the promoting efficacy of the monodesmosides on the rectal absorption of ABPC was not influenced by osmolality.\textsuperscript{21} In the present study, the effect of osmolality on the function of saponin A as a promoter for small intestinal absorption of ABPC was investigated in the in situ loop method. As shown in Fig. 5, osmotic pressure did not influence the value of \( C_{\text{max}} \) and \( AUC_{0-120} \) of ABPC by saponin A, which was similar to the results obtained for rectal absorption.

2. Effect of \( Ca^{2+} \) Ion on the Absorption Promoting Function of Monodesmosides — \( Ca^{2+} \) and \( Mg^{2+} \) play an important role\textsuperscript{18,19} in maintaining the structure of the mucous layer lining on the epithelial surface of small intestines and rectum. It has been also reported that removal of \( Ca^{2+} \) and \( Mg^{2+} \) from the mucous layer on the epithelial membrane by chelating agents such as ethylenediaminetetraacetic acid (EDTA) resulted in an increase of permeability of the membrane to drugs.\textsuperscript{20} We also showed that the enhanced rectal absorption of ABPC by \( N \)-acylcollagen-peptide,\textsuperscript{4} bile acids\textsuperscript{6} and monodesmosides\textsuperscript{2} were markedly suppressed by the addition of \( CaCl_2 \) in the solution administered. \( Ca^{2+} \) sequestration ability of each monodesmoside was determined with a dye indicator method at pH 10, because of the extremely low solubility of monodesmoside at pH 6.5. Bound \( Ca^{2+} \) of monodesmosides were 62.6 mg eq/g for saponin A, 200.4 mg eq/g for saponin B and 87.7 mg eq/g for saponin C. The effect of \( Ca^{2+} \) on the small intestinal absorption promoting function of monodesmosides was also studied using the in situ intestinal loop method. As shown in Figs. 6 and 7, the activity of saponin A to promote absorption of ABPC through small intestines was suppressed by the addition of \( CaCl_2 \) in the solution which was introduced into the small intestinal loop.

Similar results were observed with saponins B and C. However, the magnitude of the suppression was greater in the small intestine compared to that reported previously\textsuperscript{2} for the rectum. In the small intestine, the addition of 0.05% \( CaCl_2 \cdot 2H_2O \) reduced the enhanced level of ABPC in blood to the control level (Figs. 6 and 7), while in the rectum, 0.1% \( CaCl_2 \cdot 2H_2O \) showed no influence on the level of ABPC in

**FIG. 4. Enhanced Intestinal Absorption of β-Lactam Antibiotics in the Presence of Saponin A**

Dose: antibiotics 50 mg/kg, saponin A 2 mg/kg. Vol. of administration: 2.5 ml/kg. Control contains an antibiotic and XY-mix; saponin A was solubilized by XY-mix. Each point represents the mean ± S.E. of 4—6 rats. Significantly different from control, a) \( p < 0.01 \).

**FIG. 5. Effect of Osmotic Pressure on the Absorption Promoting Action of Saponin A for ABPC**

Dose: ABPC 50 mg/kg, saponin A 2 mg/kg. Vol. of administration: 2.5 ml/kg. Each point represents the mean ± S.E. of 4—6 rats.
FIG. 6. Effect of Concentration of Ca$^{2+}$ in the Solution Administered into the Loop on the Absorption Promoting Effect of Saponin A

Dose: ABPC 50 mg/kg; saponin A 2 mg/kg. Vol. of administration: 2.5 ml/kg. Each point represents the mean ± S.E. of 4–6 rats. Significantly different from the values without CaCl$_2$, a) $p<0.01$.

FIG. 7. Effect of Ca$^{2+}$ on the Enhanced Intestinal Absorption of ABPC by Saponin A, B and C

Dose: 50 mg/kg; saponins: 2 mg/kg; CaCl$_2$·2H$_2$O 0.5%. Vol. of administration: 2.5 ml/kg. Control, ABPC + XY-mix; □, ABPC + each of the saponin solubilized by XY-mix; ■, ABPC + each of the saponin solubilized by XY-mix + CaCl$_2$. Each point represents the mean ± S.E. of 4–6 rats. Significantly different from the values without CaCl$_2$, a) $p<0.01$.

blood increased by the presence of saponin A. The difference in the magnitude of suppression by CaCl$_2$ between the small intestine and the rectum is not clear. However, if calcium ion sequestration by monodesmosides in the epithelial membrane plays a role in absorption promoting action of monodesmosides, then the difference may be attributed to the difference in sequestration capacity based on the pH difference in the small intestine and in the rectum. Further study should be made to elucidate the differences.

The effect of Ca$^{2+}$ on the absorption promoting function of monodesmosides suggests a contribution of the Ca$^{2+}$ sequestration ability of monodesmosides in their action mechanisms. However, a clear mechanism is yet to be clarified.

REFERENCES

1) A part of this work was presented at the 102nd Annual Meeting of the Pharmaceutical Society of Japan, Osaka, 1982.


