PHARMACOLOGICAL STUDIES OF GARDENIA FRUIT. III. 1) RELATIONSHIP BETWEEN IN VIVO HYDROLYSIS OF GENIPOSIDE AND ITS CHOLERETIC EFFECT IN RATS

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(Received November 7, 1977)

The role of genipin in the choleretic action of geniposide, the principle component of gardenia fruit, was examined. Geniposide showed a delayed and prolonged action by intraduodenal administration of 1 g/kg or over, while genipin showed a fast choleretic action with 50 mg/kg. Geniposide did not show choleretic action after intraportal administration of 10—400 mg/kg but genipin showed a choleretic action with 2.5 mg/kg. Geniposide and genipin appeared in portal blood after intraduodenal administration of geniposide, while genipin appeared after intraduodenal administration of genipin. Geniposide and genipin glucuronide appeared in the bile after oral and intraduodenal administration of geniposide and oral administration of genipin. Genipin and genipin glucuronide that appeared in portal blood and bile after administration of geniposide and genipin showed a periodical pattern similar to that of choleretic action of these two substances. These evidences indicate that the choleretic action of geniposide is due to genipin formed by hydrolysis of geniposide in the digestive tract.

Keywords — Gardenia fruit; Gardenia jasminoides L.; geniposide; genipin; genipin glucuronide; choleretic action; in vivo hydrolysis of geniposide; intraportal injection; drug determination; and intestinal absorption.

We have, earlier, made examinations on the choleretic action of geniposide, the iridoid glucoside component contained in fruit of Gardenia jasminoides L. (San-shi-shi in Japanese), and genipin, its aglucone, in the rat and reported that genipin accelerated bile secretion to about the same degree as sodium dehydrocholate.1,2)

It was also suggested that there is a possibility that genipin may be formed by in vivo hydrolysis of geniposide in the rat.

Considering that the choleretic action may be responsible for the therapeutic effect of the gardenia fruit and that genipin is not present in this fruit, further detailed examination was made on the choleretic action of geniposide and its hydrolysis in vivo, relative to intestinal absorption and excretion into bile. Some considerations were also made on the relationship between hydrolysis in vivo and choleretic action of geniposide.

MATERIALS AND METHODS

Animals — Male Wistar strain rats weighing 200—250 g were used.

Materials — Both geniposide and genipin were prepared by the method reported previously.1,2) Each was dissolved in physiological saline or distilled water just before the administration. Volume for one-shot injection was set at 0.2 ml/100 g body weight.

Enzymes and Chemicals — β-Glucuronidase (β-D-glucuronide glucuronosohydrolase, EC 3.2.1.31) and β-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21) were purchased from Miles Laboratories, sodium dehydrocholate from Dainippon Pharmaceutical Co., and glucono-1,5-lactone from Wako Pure Chemical Ind..

Methods — (1) Choleretic Action of Geniposide and Genipin by Intraduodenal Administration: (a) Without Return of Excreted Bile
into the Body: Under anesthesia with pentobarbital sodium (50 mg/kg, i.p.), rats were laparotomized, a polyethylene cannula was inserted into the common bile duct, and the bile was collected for 1 hr. The test chemical solution was then administered into the duodenum and the bile was collected at 1-hr intervals to measure the volume of bile up to 7 hr. The control group received equivalent volume of distilled water and during the experiment rats were kept under anesthesia and were not given any supplemental solution. Sodium dehydrocholate was used as the standard substance.

(b) With Return of Excreted Bile into the Body: The rats were laparotomized and the bile ductular cannula and intraduodenal cannula were provided, and the bile was collected twice at 30-min intervals. The test chemical was administered into the duodenum and the bile was collected at 30-min intervals to measure the volume of bile up to 6 hr. The collected bile was returned into the duodenum through the inserted cannula each time after measurement of the volume. The control group received equivalent volume of distilled water and during the experiment rats were kept under anesthesia and were not given any supplemental solution. Sodium dehydrocholate was used as the standard substance.

(2) Choleretic Action of Geniposide and Genipin by Intraperoral Administration: The rat was treated as in (1), the bile was collected for 1 hr, and an injection needle attached with a polyethylene cannula was proximally inserted into the portal vein and fixed with an adhesive (Aron-alpha, Toa Gosei Kagaku) without disturbing the portal blood flow. The other end of the cannula was connected to an injection syringe provided with an automatic injection apparatus (KN-202, Natume Seisakusho), and geniposide was injected immediately after insertion of the cannula, at the rate of 10 ml/hr for 15 min. In the case of a low dose of geniposide and any dose of genipin, administration was made by a one-shot injection. The bile was collected at 1-hr intervals for 6 hr in the case of geniposide and at 15-min intervals up to 60 min in the case of genipin to measure the volume of bile. The control group was given equivalent volume of physiological saline at the same rate.

(3) Absorption of Geniposide and Genipin from Digestive Tract after their Intraduodenal Administration (In situ Preparation): (a) Separation and Determination of Genipin and Geniposide from Portal Blood after Administration of Geniposide: The rats were treated as in (1)(a) and the bile was drained. Geniposide (2 g/kg) was injected into the duodenum and 2,3,4,5, and 6 hr after its administration, a cannula was distally inserted in the portal vein with ligation and immediately portal blood was collected for 30 min using different animals for each time period. At the time of portal blood collection, the same volume of heparinized blood was transfused from the femoral vein (0.83 ml/min). The collected blood was centrifuged (3000 rpm, 10 min) immediately, the plasma was treated as described in (5)(a), and geniposide and genipin in the plasma were separated and determined.

(b) Separation and Determination of Genipin from Portal Blood after Genipin Administration: The rats were treated as in (3)(a), 50 mg/kg of genipin was injected into the duodenum and the portal vein was immediately cannulated similarly as in (3)(a). The portal blood from the cannula was collected three times at 15-min intervals, treated as above, and the amount of genipin in the plasma was determined. In this case also, heparinized blood was transfused at the same rate as that of the blood withdrawn during the experiment.

(4) Separation and Determination of Genipin and Genipin Glucuronide from the Bile of a Rat given Geniposide or Genipin: (a) Oral or Intraduodenal Administration: After oral administration of geniposide or genipin, the rats were treated as in (1), except that the chemical was administered intraduodenally after the operation in the case of duodenal administration. The bile was collected beginning 30 min after oral or intraduodenal administration of 2 g/kg of geniposide, for a total of 9 times at 1-hr intervals. After intraduodenal administration of 100 mg/kg of genipin, the bile was collected three times at 30-min intervals beginning 15 min after the administration, and then twice at 1-hr intervals. The
bile was treated as described later, and genipin per se and that derived from genipin glucuronide were separated and determined.

(b) Intraperitoneal Administration: The bile collected in Method (2) was treated as described below, and genipin per se and that derived from genipin glucuronide were separated and determined.

(5) Separation and Determination of Genipin, Geniposide, and Genipin derived from Genipin Glucuronide in Biological Materials (Bile and Plasma): (a) Genipin: As shown in Chart 1, a and b, bile or plasma sample was extracted three times with ether, the extract was concentrated, and submitted to preparative thin-layer chromatography (TLC). The fraction with the same Rf value as genipin detected under an ultraviolet (UV) lamp (2536 Å) was scraped off and extracted 5 times with warm ether. The extract was evaporated and thoroughly dried with a vacuum pump, and the residue was trimethylsilylated by the addition of trimethylsilylating reagent. To this reaction mixture was added 50 μg of 2-acetoamido-5-chlorobenzophenone as an internal standard, mixed thoroughly, dried in N2 stream, and extracted with CHCl3. The CHCl3 solution was submitted to gas-liquid chromatography (GLC). The quantity of genipin was obtained from the peak area in GLC, h×half-width, in comparison with the peak area of the internal standard, and calculated from the calibration curve. Recovery of genipin by the present method was 90.65% (S.D.: 1.38). In the present experiments, the data are uncorrected.

For the preparative TLC, silica gel plate (Kieselgel HF254, Merck) was prepared in 0.5 mm thickness and activated at 105° for 1 hr. The plate was developed in ether.

For the trimethylsilylation, the dried sample was dissolved in 3 drops of pyridine, 4 drops of hexamethyldisilazane and 2 drops of trimethylchlorosilane were added, and the mixture was shaken vigorously for 30 sec. GLC was carried out with Hitachi Model 073 chromatograph under the following conditions: Glass column (3 mm×2 m); column packing, silicone OV-17, 2% on Uniport Q (80/100 mesh); temperature programming, 150—280° (5°/min); carrier gas, N2 at 30 ml/min.

(b) Geniposide: As shown in Chart 1, a, the plasma or bile was extracted with ether to remove genipin, the aqueous layer was lyophilized (dry ice-acetone) and the dried product was refluxed with MeOH for 30 min. This MeOH extract was concentrated to 5 ml and 20 μl of this extract was spotted on a commercial TLC plate (Merck's Silicagel plate F254, 20X20 cm, 0.5 mm thickness). The plate was developed to 10 cm with CHCl3: MeOH (3:1) and the spot of geniposide was determined by a thin-layer densitometer, under the following conditions: Dual-wavelength and zigzag scanning type densitometer (Model CS900, Shimadzu Seisakusho); wavelength, λs = 240 nm, λk = 350 nm; linearization program, (A) SX = 3 (linearizer, LIN-53, Shimadzu Seisakusho). Recovery of geniposide by the present method was 89.61% (S.D.: 2.68). In the present experiments, the data are uncorrected.

(c) Geniposide derived from Genipin Glucuronide: (i) Examination of Incubation Time: After intraduodenal administration of 50 mg/kg of genipin, 14 ml of bile was collected from the rats (10 heads), 2 ml was placed in each of test tubes, and one test tube was submitted to the measurement of genipin by the procedure shown in Chart 1.

To the remaining test tubes, 2 ml of phosphate buffer (pH = 6.9) containing 1% β-glucuronidase was added. The tubes were incubated for 1, 2, 3, 4, 6, or 8 hr, respectively. The amount of genipin formed after the incubation was determined. After this determination, the residual ether in the mother liquor was removed as much as possible, then, β-glucuronidase was added to each tube, and the tubes were incubated for 2 hr to see if genipin glucuronide still remained in the test tube.

(ii) Method of Determination: As shown in Chart 1, b, the bile was extracted with ether to remove genipin. After residual ether was removed from the aqueous layer as much as possible, approximately equal volume of phosphate buffer containing 1% β-glucuronidase was added and the mixture incubated at 37° for 2 hr.

In case of the bile from a rat given geniposide,
ca. 500 mg of glucono-1,5-lactone was added to each sample before incubation. The incubated mixture was processed in the same way as described above for the determination of genipin. Statistical Analysis: Statistical analysis was made using Student's $t$ test.

RESULTS

1. Choleretic Action after Intraduodenal Administration of Geniposide and Genipin

(a) Without Return of Excreted Bile into the Body —— This result is shown in Fig. 1. In the control group, flow of the bile decreased with time while in the animals given geniposide, significant increase in bile flow was noted, 5 hr after administration of 1 g/kg and 2 hr after 2 g/kg. A choleretic effect comparable to 50 mg/kg of sodium dehydrocholate was observed after administration of 50 mg/kg of genipin.

(b) With Return of Excreted Bile into the Body —— The result of this experiment is shown in Fig. 2. In the control group, approximately constant level of bile flow was observed during the experimental period by the present procedure. A significant and enduring choleretic effect was seen with geniposide, beginning at 4 hr after administration of 1 g/kg and 1.5 hr with 2 g/kg. Genipin and sodium dehydrocholate showed about the same action as seen in (a).

2. Choleretic Action of Geniposide and Genipin after Intraperitoneal Administration

Geniposide failed to show any choleretic action with 10—400 mg/kg, but 2.5 mg/kg of genipin showed a significant choleretic activity, reaching the maximum 15 min after the administration. This result is shown in Fig. 3.

3. Absorption of Geniposide and Genipin from Digestive Tract after their Intraduodenal Ad-
Choleretic Effect of Geniposide

FIG. 1. Choleretic Action of Geniposide and Genipin in Rats

- : control (n = 11),
○ : geniposide 1000 mg/kg (n = 9),
□ : geniposide 2000 mg/kg (n = 17),
× : genipin 50 mg/kg (n = 8),
△ : dehydrocholate-Na 50 mg/kg (n = 5).
*: p<0.05, **: p<0.01, ***: p<0.001.
i.d.: intraduodenal administration.
Excreted bile was not returned into the body.

FIG. 2. Choleretic Action of Geniposide and Genipin in Rats

- : control (n = 10),
○ : geniposide 1000 mg/kg (n = 10),
□ : geniposide 2000 mg/kg (n = 10),
× : genipin 50 mg/kg (n = 10),
△ : dehydrocholate-Na 50 mg/kg (n = 10).
*: p<0.05, **: p<0.01, ***: p<0.001.
i.d.: intraduodenal administration.
Excreted bile was returned into the body.

FIG. 3. Choleretic Action of Genipin in Rats

- : control (n = 11),
○ : genipin 2.5 mg/kg (n = 6),
× : genipin 5.0 mg/kg (n = 6),
△ : genipin 10.0 mg/kg (n = 6).
*: p<0.05, **: p<0.01.
i.p.v.: intraportal administration.
Vertical bars indicate S.E.

FIG. 4. Appearance of Geniposide and Genipin in Portal Blood after Intraduodenal (i.d.) Administration of Geniposide in Rats

○ : geniposide,
× : genipin.
a): mean ± S.E.
Numbers in the parentheses indicate the number of animals.

Genipin appeared 2 hr after administration of geniposide and its level reached a peak after 4—5 hr. The amount of genipin at the peak period (for 30 min) was ca. 0.25 mg. Geniposide was present from the initial period after administration and reached a peak after 3 hr, its blood level at the peak period (for 30 min) being ca. 0.72 mg.
(b) Separation and Determination of Genipin in Portal Blood after Administration of Genipin—Genipin was detected in portal blood after administration of 50 mg/kg of genipin, and its periodical variation is shown in Fig. 5. Genipin appeared in portal blood 15 min after its administration, the amount increased with time, and its blood level was ca. 0.14 mg at the third 15-min period after blood collection.

4. Separation and Determination of Genipin and Genipin Glucuronide from Bile of Rats given Geniposide and Genipin

(a) After Oral or Intraduodenal Administration—Both genipin and genipin glucuronide were detected in the bile after oral or intraduodenal administration of 2 g/kg of geniposide or oral administration of 100 mg/kg of genipin. Periodical variation after oral administration is shown in Fig. 6. Genipin and genipin glucuronide began to appear 1—2 hr after oral administration of geniposide, and the amount of genipin at peak period (for 1 hr) reached ca. 5 μg and that of genipin glucuronide was ca. 0.6 mg. Approximately the same result was obtained with intraduodenal administration. After oral administration of genipin, genipin and genipin glucuronide level reached the peak within 1 hr, and the amount of genipin was ca. 25 μg and that of genipin glucuronide ca. 0.65 mg, at the first collection period (for 30 min).

(b) Intraportal Administration—Geniposide was detected in the bile after administration of 400 mg/kg of geniposide into the portal vein, but neither genipin nor genipin glucuronide was detected.


Gas chromatogram of genipin from biological materials is shown in Fig. 7.

Genipin derived from Genipin Glucuronide—(i) Examination of Incubation Time: Fig. 8 shows the relationship between the amount of genipin and the period of incubation of rat bile with β-glucuronidase after genipin administration.
Choleretic Effect of Geniposide

is known to be contaminated with β-glucosidase, incubation of geniposide with β-glucuronidase was carried out and the formation of a minute amount of genipin was observed. This formation was completely suppressed by the addition of glucono-1,5-lactone, the specific inhibitor of β-glucosidase.

DISCUSSION

Both geniposide and genipin increased bile secretion by intraduodenal administration but the pattern of their action was different. Genipin showed a choleretic action in the initial period after administration of 50 mg/kg, as was the case in our previous work, but geniposide showed a prolonged choleretic activity at 4 hr after administration of 1 g/kg and 2 hr with 2 g/kg. In the intraportal administration, genipin showed a choleretic action at a dose of 1/20 to 1/10 that of intraduodenal administration, while geniposide did not show any action at doses of 10—400 mg/kg.

In the absorption experiment, genipin appeared in the portal blood in the early phase after intraduodenal administration of 50 mg/kg of genipin. After intraduodenal administration of 2 g/kg of geniposide, geniposide and genipin appeared in portal blood; geniposide appeared in the early phase after the administration and reached a peak 3 hr later, while genipin began to appear gradually from 2 hr later and reached a peak 4—5 hr after administration. These findings show that genipin is absorbed per se from the digestive tract and transported to the liver, while geniposide is absorbed in the initial period after the administration but a part of it is hydrolyzed in the digestive tract, absorbed in the form of genipin, and transported to the liver. Comparison of the choleretic action of these chemicals after intraduodenal administration and data of absorption studies indicate that the choleretic pattern of geniposide and genipin is similar to the pattern of genipin absorbed into the portal blood after administration. These studies indicate that the choleretic action of geniposide is due to genipin formed by its hydrolysis.

Genipin and genipin glucuronide appeared in the bile after intraduodenal administration of geniposide and genipin. Genipin and genipin
glucuronide appeared in the early period after administration of 100 mg/kg of genipin, while they began to appear 1—2 hr after administration of 2 g/kg of geniposide, and reached a peak 4—8 hr later. The patterns of their appearance were similar to the absorption pattern of genipin after administration of the two agents in absorption experiments. In addition, as shown above, intraportal administration of 10—400 mg/kg of geniposide failed to show any choleretic activity. In this case, geniposide appeared in the bile but not genipin or genipin glucuronide. When geniposide and genipin are injected into the digestive tract, a part of genipin absorbed into the portal vein undergoes conjugation with glucuronic acid in the liver and is excreted into the bile, together with a minute amount of non-conjugated genipin. Geniposide absorbed into the portal vein is not hydrolyzed in the liver and at least a part of it is excreted *per se* into the bile.

In conclusion, when gardenia fruit containing geniposide is ingested, geniposide is hydrolyzed in the intestine and genipin formed is absorbed in portal blood, together with unchanged geniposide. The genipin absorbed shows a choleretic action in the liver, at least a part of it undergoes conjugation with glucuronic acid, and a minute amount of genipin is excreted into the bile in the free form. Geniposide absorbed unchanged is not hydrolyzed in the liver and a part of it is excreted in bile unchanged, without exerting a choleretic action. Considering the intestine—liver circulation, a part of geniposide excreted into the bile may undergo hydrolysis in the digestive tract and take part in the prolonged choleretic action.

**Acknowledgements**  We are grateful to the late Dr.T.Kariyone, Dr. I.Yoshioka and Dr.H.Taguchi, Tsumura Laboratory, for their pertinent advice and suggestions.

**REFERENCES**

