Simultaneous Estimation of Geniposide and Genipin in Mouse Plasma Using High-Performance Liquid Chromatography

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In Japan, gardenia fruit (fruit of Gardenia jasminoides, Rubiaceae) has been utilized as a herbal medicine and a source of natural pigment; it has also been used for external fomentation and has been blended in Chinese medicinal prescriptions. The representative components, excluding pigments, in this crude drug are iridoid glycosides; 1–4 one of these, geniposide, 5,6 is the principal constituent. It has been reported that geniposide is transformed to genipin 7,8 by intestinal bacteria in animals, and that genipin shows several biological activities. 9–11 Published papers have reported on the quantitative determinations of geniposide and related iridoids in this crude drug by using thin-layer chromatography 12 or high-performance liquid chromatography (HPLC). 13–19

In the present work, we attempted a simultaneous estimation of geniposide and its aglycon, genipin, in mouse plasma after oral administration of hot water extract of Gardenia fruit using HPLC with a column-switching system.

Experimental

Materials
Chopped Gardenia fruits were obtained from Nakai-kohshindo (Kobe, Japan). The reagents and solvents for HPLC and authentic geniposide were purchased from Nacalai Tesque (Kyoto, Japan). Authentic genipin was provided by Jujo Chemical (Tokyo, Japan). Human plasma was obtained from Cosmo Bio (Tokyo, Japan).

Animals
Male ddY mice (from Nihon SLC, Hamamatsu, Japan) weighing between 25–30 g were used. The animals were housed in plastic cages with free access to food (until 12 h before use) and water, and were kept in a room at 25 ± 1˚C, 55 ± 5% humidity with a 12 h dark-light cycle.

Extract of Gardenia fruit
Crude drug was refluxed (for 1 h) twice with a five-fold volume of water and filtered, followed by removal of the combined solvent in vacuo. The extract was dried in vacuo (the content of geniposide, 180.3 mg/g) and the residue was dissolved in distilled water. The extract solution was given orally to mice at a constant injection volume of 0.6 ml/30 g body weight.

Collection of blood plasma
After each administration of 1200 mg/kg of Gardenia fruit extract (corresponding to 216.4 mg/kg of geniposide), cardiac blood was collected from mice using a heparin-treated cylinder. The plasma was immediately separated by centrifugation and used for analysis.

High-performance liquid chromatography
The experimental conditions are described below. The equipment used were as follows: a high-pressure machine (3001 Inert pump (Shiseido)), a detector (3002 UV-VIS spectrophotometer (Shiseido, wavelength 240 nm, 0.001 AUFS)), columns (Capcell Pak MF C18 (Shiseido, 4.6 mm i.d. × 50 mm, for pretreatment), Capcell Pak C18 UG120 (Shiseido, 2.0 mm i.d. × 35 mm, for concentration of samples), Capcell Pak C18 MG (Shiseido, 1.5 mm i.d. × 250 mm, for final separation)). The mobile phases were 100 mM phosphate buffer (pH 6.8)-acetonitrile (98:2, for pretreatment and concentration of samples); 100 mM phosphate buffer (pH 6.8)-acetonitrile (85:15, for final separation). The flow rates were 200 µl/min (for pretreatment and concentration of samples); 100 µl/min (for final separation). The column temperature was 40˚C. The column switching times were set to 3 min, 30 s (pretreatment → concentration of samples) and 8 min, 30 s (concentration of samples → final separation), respectively. The chromatograms were recorded on a SIC Chromatocorder 21 (chart speed, 0.5 cm/min) and the peak areas were compared for quantitative determinations.

Determination of geniposide and genipin
After the administration of Gardenia fruit extract to a mouse, phosphoric acid was added to the separated plasma (20 µl/ml), followed by centrifugation in Ultrafree-MC (Millipore) at 9000 rpm for 30 min. Then, 25 µl of filtrate was subjected to HPLC. The geniposide and genipin contents were estimated based on the peak areas (n = 3).

Results and Discussion
Many papers have reported on the utility of the column-switching technique for the determination of drugs in blood. 20–25 Analyses of geniposide and genipin together with other iridoids have already been reported. 17 However, there have been no reports published regarding the detection and separation of these two compounds from each other in plasma from an animal. When the HPLC conditions were employed as described in the

Notes

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experimental section, geniposide and genipin in mouse plasma were detected and separated well within 30 min. A chromatogram of the plasma 90 min after oral administration of a hot-water extract of Gardenia fruit (1200 mg/kg) to mouse is shown in Fig. 1. As concurrently shown in Fig. 1, without column switching, the chromatogram was complicated by numerous useless peaks owing to a difficulty to exclude unrelated components in the plasma; the genipin peak was smaller because of its lack of any concentration effect. The detection limit was about $1.2 \times 10^{-4}$ $\mu$g (geniposide) and $1.5 \times 10^{-4}$ $\mu$g (genipin).

The application of this analytical condition to estimate the geniposide and genipin levels in mouse plasma was then examined. Calibration curves for the geniposide (0.1375 – 5.5000 $\mu$g, dissolved in plasma) and the genipin (0.0013 – 0.0070 $\mu$g, dissolved in plasma) were prepared, and regression equations were derived from the least-squares method, as follows: $y = 7.4198 \times 10^{-8} x + 0.01561$ ($r = 0.999$, for geniposide); $y = 2.7464 \times 10^{-8} x + 0.0005414$ ($r = 0.999$, for genipin). Here, $x$ is the peak area expressed as the count number on the chromatogram and $y$ is the amount of the compound ($\mu$g). The changes in the geniposide and genipin concentrations in mouse plasma after oral administration of 1200 mg/kg hot-water extract of Gardenia fruit are shown in Figs. 2 and 3. Because the content of geniposide in the extract was 180.3 mg/g, this dosage can be converted to a direct dose of 216.4 mg/kg geniposide. In contrast, the content of genipin in the extract was a trace amount. The plasma geniposide level reached a maximum at 30 min (about 103.1 $\mu$g/ml) and gradually decreased, while a very small amount of genipin was detected in the plasma; its concentration seemed to peak at 60 min (about 0.07 $\mu$g/ml). It is already known that geniposide is hydrolyzed to genipin in the intestine of an animal.9-11 Based on the estimation method and result described in this paper, a considerable amount of geniposide appeared to pass into the blood. On the other hand, its aglycon, genipin, is known to be a labile substance that reacts with amino acids and proteins to form a blueish coloring matter.7,8 Consequently, these

Fig. 1 Elution profiles of mouse plasma obtained by HPLC after the oral administration of an extract of Gardenia fruit. The analytical conditions are described in the experimental section. Peaks: 1, geniposide; 2, genipin. a, after administration of extract (90 min) without column-switching system; b, without administration of extract; c, after administration of extract (90 min).

Fig. 2 Change in the geniposide concentration in mouse plasma after the oral administration of an extract of Gardenia fruit. Each point represents the mean±S.E. (n = 3). The vertical lines show the standard error of the mean. Dosage, 1200 mg/kg.

Fig. 3 Change in the plasma genipin level after the oral administration of an extract of Gardenia fruit to mice. Each point represents the mean±S.E. (n = 3). The vertical lines show the standard error of the mean. Dosage, 1200 mg/kg.
properties may contribute to the extremely small amount of genipin passed into blood after hydrolysis.

Furthermore, a chromatogram of human plasma after the addition of authentic geniposide and genipin is shown in Fig. 4. It can be presumed that the concentrations of both compounds in human blood are capable of being estimated by the same analytical conditions. Hence, the availability of a method for determining the geniposide and genipin levels in human blood after taking doses of Gardenia fruit or Chinese medicinal prescriptions containing Gardenia fruit is suggested.

Using HPLC with a column-switching system, simultaneous estimations of both compounds in blood were possible. Also, there were advantages of less deterioration of the column and improved sensitivity and precision.

References
