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Note

Determination of glycyrrhizin and its metabolite glycyrrhetinic acid in rabbit plasma by high-performance liquid chromatography after oral administration of licorzin

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Licorzin is a new drug that constists of zinc and effective components extracted from the root of glyrrhiza The root extract reacts with inorganic zinc to form a precipitate, which is named licorzin The content of inorganic zinc ion and glycyrrhizin (G) in licorzin are 4–5% and 25%, respectively Besides overcoming zinc deficiency in children the drug has been exploited for the treatment of gastric ulcers, duodenal ulcers and stomatocace by oral administration G and its metabolite glycyrrhetinic acid (GA) both show anti-ulcer action [1] Therefore, a method for the determination of G and GA in plasma is necessary in order to investigate the absorption of licorzin and the elimination of G and GA

The analysis of G in glyrrhiza root has been reported [2,3]. Quantitative analysis of G and GA in biological materials has been performed by capillary gas chromatography with selected-ion monitoring [4], high-performance liq-

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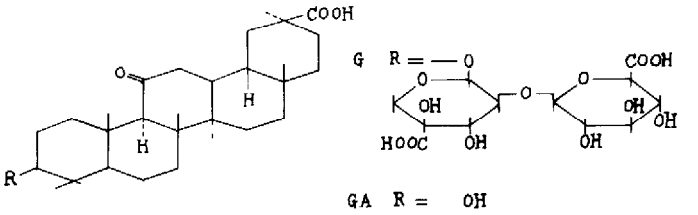

Fig 1 Structures of glycyrrhizin (G) and glycyrrhetinic acid (GA)

uid chromatography (HPLC) [5] and enzyme immunoassay [7,8] Sakiya et al [9] have described an HPLC method for the simultaneous determination of G and GA using a strongly basic ion-exchange resin column and a linear gradient system after oral administration of G to rats Ichikawa et al [10] found that G and GA in plasma can be determined by HPLC using two kinds of mobile phase system after G and GA were injected intravenously into rats, although the sample pretreatment was time-consuming Until now, no method has been reported for the determination of G and GA in plasma after oral administration of licorzin to rabbits

G and GA differ greatly in polarity (Fig 1) so we have studied the reversed-phase HPLC determination of G and GA in plasma by using two kinds of mobile phase system. The pretreatment of plasma samples is very simple The sample solution for HPLC can be obtained in a single pretreatment step and may be used to determine both G and GA The method proposed was also applied to determine G and GA plasma concentrations after oral adminstration of licorzin to rabbits.

EXPERIMENTAL

Chemicals

Methanol, acetonitrile, glacial acetic acid and propylparaben (PPB) were of analytical grade Water was redistilled Licorzin, with a G content of 25% and a GA content of less than 0 3%, was produced by Xinjiang Pharmaceutical Factory (XPF, China) G standard was provided by XPF and standard GA by Huhehaota Pharmaceutical Factory (China)

Apparatus and chromatographic conditions

The apparatus used was a Shimadzu high-performance liquid chromatograph, Model LC-4A, with a UV detector operating at 256 nm and a Nucleosil C_{18} column (250 mm × 4 6 mm I D , 5 μm particle size) The mobile phase was acetonitrile–water–acetic acid (38 62 0 5, v/v) for G and methanol–water–acetic acid (83 17 0 5, v/v) for GA The column was maintained at 20–25 °C, and the mobile phase flow-rate was 1 0 ml/min

Sample preparation

Plasma samples (1 ml) were diluted with 5 ml of methanol containing the internal standard (PPB) (0 8 μg), mixed for 1 min, and then centrifuged for 5 min at 1250 *g*. Then 4.5 ml of the supernatant liquid were dried at room temperature under an air stream The residue was dissolved in 0.4 ml of methanol and filtered through a Millipore membrane filter (FH 0.5 μm pore size) to obtain ca 200 μl of filtrate, which was used for the HPLC determination of G and GA. a 50-μl aliquot was injected each time

Calculation

G was calculated by the method of peak height of internal standard (PPB) and GA by the method of peak height of external standard (there is no suitable internal standard for GA)

RESULTS AND DISCUSSION

Eluents and chromatograms

G and GA differ greatly in polarity so two different mobile phases were used to determine G and GA in plasma. We eventually selected a mobile phase of acetonitrile–water–acetic acid (38 62 0 5, v/v) for G Some impurities in plasma, G and PPB can be separated using the mobile phase (Fig 2) without the appearance of the GA peak. Methylparaben (MPB), PPB and butylparaben (BPB) were tested as internal standards The MPB peak overlapped with the peaks of the solvent and impurities in the plasma. The retention time of BPB was too long (25 min) and the peak of BPB was too broad Only PPB was an ideal internal standard for G The pretreated plasma sample was injected for HPLC after the oral administration of licorzin to rabbits. The retention time of G was prolonged or shortened following a change of the proportion of water in the mobile phase, but the peak was still a single one.

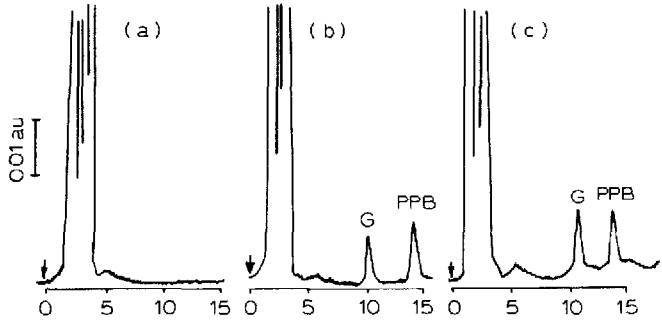

Fig 2 Chromatograms of rabbit plasma (a) Drug-free plasma, (b) plasma spiked with G and internal standard (PPB), (c) extracted plasma sample after oral administration of licorzin (1 5 g/kg) The eluent was acetonitrile–water–acetic acid (38 62 0 5, v/v)

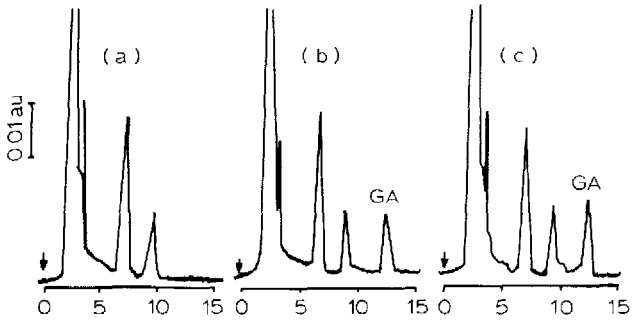

Fig 3 Chromatograms of rabbit plasma (a) Drug-free plasma, (b) plasma spiked with GA, (c) extracted plasma sample after oral administration of licorzin (1 5 g/kg) The eluent was methanol-water-acetic acid (83 17 0 5, v/v)

The mobile phase of methanol–water–acetic acid (83 17 0.5, v/v) was used to separate the peaks of GA, G, PPB and impurities in plasma (Fig. 3) G and PPB were eluted almost together with this mobile phase We did not find a suitable internal standard for GA

Linearity and precision

Standard G, GA and internal standard PPB were added to 1 ml of drug-free plasma, and the sample was pretreated The peak-height ratio of G to internal standard was found to be linear throughout the range 1–20 μg/ml (r=0 9993) The linearity range of the peak height of GA was 0.5–20 μg/ml (r=0 9992). The results of precision studies are presented in Table I The inter-assay and intra-assay coefficients of variation (C.V.) were all less than 7%

Recovery

The recovery of the method was evaluated by analysing plasma samples containing known amounts of G, GA and PPB The mean recoveries of G and GA were 83 4 $\pm$ 2 0 and 94 9 $\pm$ 4 3%, respectively (Table II)

Plasma concentration of G and GA after oral administration to rabbits

Four healthy rabbits weighing 2.0–2 5 kg (male and female) were used. Blood was drawn pre-dose and at approximately the following times 4, 8, 12, 14, 16, 18, 22, 27, 32 and 38 h after oral administration of licorzin (1 5 g/kg) About 2 ml of blood were drawn through the auricular vein and collected in heparinized tubes. Immediately after sampling, the blood samples were centrifuged for 5 min at 1250 g to obtain 1 ml of plasma, which was subjected to analysis for G and GA. The mean plasma concentration–time curves of G and GA are shown in Fig. 4. The peak time of G was ca 14 h, and that of GA was 22 h. The peak concentration of GA (ca. 2.2 μg/ml) was less than that of G (ca. 7 μg/ml) These results indicate that in rabbits GA is the main metabolite of G, because

TABLE I

PRECISION AND ACCURACY

Drug	Amount added (μg/ml)	Amount found (mean ± S D , $n=10$) (μ/ml)	Precision (C V) (%)	Accuracy (%)
Intra-assay				
G	1 00	1 06 ± 0 07	6 6	106
	2 00	1 99 ± 0 12	6 0	99 5
	5 01	4 95 ± 0 22	4 4	98 8
	10 02	10 14 ± 0 35	3 5	101
GA	0 50	0 51 ± 0 03	5 9	102
	2 02	1 90 ± 0 07	3 8	94 1
	5 04	5 13 ± 0 20	4 0	101
	10 08	9 30 ± 0 26	2 7	92 3
Inter-assay				
G	1 00	1 04 ± 0 07	6 7	104
	2 00	2 01 ± 0 11	5 5	100
	5 01	4 85 ± 0 21	4 3	96 8
	10 02	9 96 ± 0 43	4 4	99 4
GA	0 50	0 50 ± 0 03	6 0	100
	2 02	2 05 ± 0 11	5 4	101
	5 04	5 05 ± 0 18	3 5	100
	10 08	9 64 ± 0 36	3 7	95 6

TABLE II

RECOVERY OF G AND GA

Drug	Amount added (μg/ml)	Amount found (mean ± S D , $n=3$) (μg/ml)	Recovery (%)		C V (%)
			Individual values	Mean ± S D	
G	1 00	0 84 ± 0 05	84 0	83 4 ± 2 0	2 4
	2 00	1 73 ± 0 06	86 4		
	5 01	4 07 ± 0 04	81 2		
	10 02	8 20 ± 0 06	82 0		
GA	0 50	0 51 ± 0 09	102 0	94 9 ± 4 3	4 5
	2 02	1 90 ± 0 03	94 2		
	5 04	4 59 ± 0 12	92 1		
	10 08	9 19 ± 0 08	91 2		

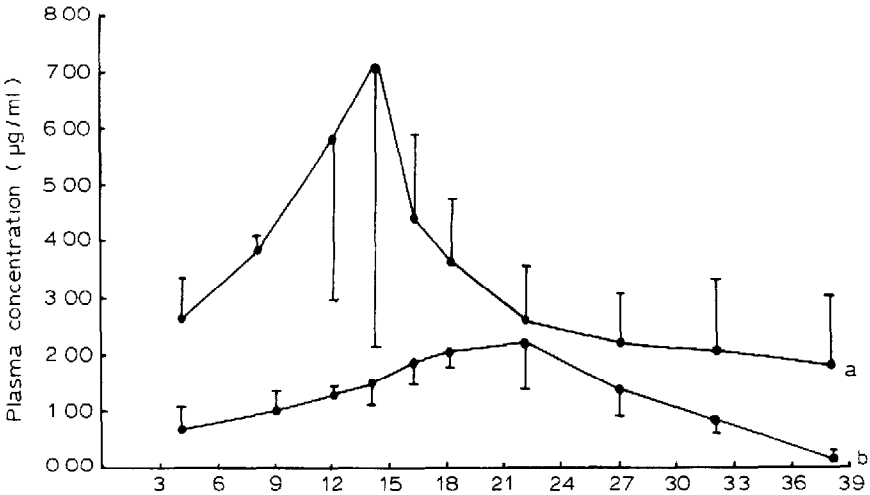

Fig 4 Mean plasma concentration-time curves of (a) G and (b) GA after oral administration of licorzin (1 5 g/kg) in four rabbits

there is nearly no GA in licorzin (less than 0.3%) Also the results suggest that G may be absorbed in two forms, the parent form (G) and its metabolite (GA).

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