Inhibitory Effects of Glycyrrhetic Acid Derivatives on 11β- and 3α-Hydroxysteroid Dehydrogenases of Rat Liver

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Glycyrrhetic acid (GA), an aglycone of glycyrrhizin (GL), is a potent inhibitor of 11β- and 3α-hydroxysteroid dehydrogenases. 11β-Hydroxysteroid dehydrogenase activity of rat liver microsomes was potently inhibited by GA, 3-deoxyglycyrrhetic acid (3-deoxyGA), 3-ketoglycyrrhetic acid (3-ketoGA), 3-epiglycyrrhetic acid (3-epiGA) and 11-deoxoglycyrrhetic acid (11-deoxoGA), with \( I_{50} \) values of 2–4 \( \times 10^{-7} \) M. However, 18α-stereoisomers (\( I_{50} = 3–7 \times 10^{-6} \) M) of GA, 3-deoxyGA and 11-deoxoGA were one tenth less inhibitory on the enzyme activity than the corresponding 18β-isomers. On the other hand, 18α-stereoisomers of GA, 3-deoxyGA and 11-deoxoGA inhibited 3α-hydroxysteroid dehydrogenase activity of rat liver cytosol more potently than the corresponding 18β-isomers. \( I_{50} \) values of 18α- and 18β-isomers were 2 and 7 \( \times 10^{-6} \) M, respectively, in the case of GA, 8 and 20 \( \times 10^{-6} \) M in 3-deoxyGA, 3 and 20 \( \times 10^{-6} \) M in 11-deoxoGA. These results indicate that the 18β-conformation of oleanane is important for the inhibition of 11β-hydroxysteroid dehydrogenase but on the contrary the 18α-conformation is important for the inhibition of 3α-hydroxysteroid dehydrogenase.

Keywords: glycyrrhetic acid; 18α-glycyrrhetic acid; 11β-hydroxysteroid dehydrogenase; 3α-hydroxysteroid dehydrogenase;

Introduction

Glycyrrhizin (GL), an active component of licorice, Glycyrrhiza glabra, is ingested orally as a component of oriental medicine and as a sweetener. GL shows various pharmacological effects including a steroid-like action,\(^1\) anti-viral action\(^2\) and interferon-inducing activity,\(^3\) but has a side effect such as pseudoaldosteronism.\(^4,5\) On the other hand, GL is hydrolyzed to 18β-glycyrrhetic acid (GA), an aglycone of GL, by human intestinal bacteria\(^6,7\) though GL is not hydrolyzed to GA, but to GA monoglucuronide, by human liver \( \beta \)-glucuronidase.\(^8\) Moreover, when GL is administered orally to human beings, GL is not detected in their sera but GA is detected.\(^9\) Accordingly, these results suggest that GL administered orally is not absorbed from the gastrointestinal, but GA is absorbed after the hydrolysis of GL to GA by intestinal bacteria and shows various pharmacological effects. Several studies have been reported on the inhibitory effects of GL and GA on steroid-metabolizing enzymes such as 5α-reductase,\(^10,11\) 5β-reductase,\(^10,11\) 3α-hydroxysteroid dehydrogenase (3α-HSD),\(^12,13\) 3β-hydroxysteroid dehydrogenase (3β-HSD)\(^13\) and 11β-hydroxysteroid dehydrogenase (11β-HSD)\(^14\) and revealed that GA, not GL, potently inhibited the activities of 5β-reductase, 3α-HSD, 3β-HSD and 11β-HSD. Therefore, it was postulated that the inhibition of 5β-reductase, 3α-HSD, and 11β-HSD resulted in steroid-like action, anti-inflammatory action, and pseudoaldosteronism, respectively.

18α-Stereoisomer of GA (18α-glycyrrhetic acid, 18α-GA) had more potent inhibitory effects on 5β-reductase\(^11\) and 3α-HSD\(^12\) activities than GA, and also showed stronger anti-inflammatory action than GA,\(^11,13\) suggesting that the 18α-planar conformation of GA is important for these effects. On the other hand, the 11-oxo group in ring C of GA was found to be essential for the inhibition of 5β-reductase activity because 11-deoxo-derivatives had little inhibitory effects on the enzyme activity.\(^16\) Moreover, the 3β-hydroxyl group in ring A of GA seemed to be related to the inhibition of 3α-HSD activity because the activity was less inhibited by 3-epi-18β-glycyrrhetic acid (3-epiGA).\(^13\) However, the inhibitory effects of various GA derivatives on 11β-HSD activity, which give rise to the pseudoaldosteronism, have not been studied previously.

In the present paper, inhibitory effects against 11β- and 3α-HSD activities were examined with 18α- and 18β-stereoisomers of GA, 3-deoxyglycyrrhetic acid (3-deoxyGA) and 11-deoxoglycyrrhetic acid (11-deoxoGA) (Fig. 1).

Materials and Methods

Animals and Preparation of Hepatic Microsomes and Cytosol Wistar-strain male rats, 8–12 weeks old, were purchased from Japan SLC Co. (Shizuoka). After sacrifice of the rats by decapitation, livers perfused with saline were homogenized in 4 volumes of 0.15 M KCl solution, and

![Fig. 1. Structure of GA, 3-DeoxoGA and 11-DeoxoGA Symbol (●) shows 18-position of carbon.](image)
Inhibitors at varying concentrations were added in 5–20 μl of methanol or acetonitrile. Control velocity without inhibitors was determined in the presence of the corresponding quantities of organic solvent. \( I_{50} \) values were obtained from linear-regression lines as the final concentrations of inhibitors that gave 50% inhibition.

**Determination of Protein**

Protein was determined by the method of Lowry et al.\(^{13}\) using bovine serum albumin as the standard.

**Chemicals**

GA was purchased from Nakalai Tesque Inc. (Kyoto), and purified by repeated crystallization. 3-EpiGA and 3-keto-18β-glycyrrhetic acid (3-ketoGA) were prepared according to the method reported previously.\(^{61}\) 18x- And 18β-stereoisomers of 3-deoxyGA and 11-deoxoGA were synthesized as reported previously.\(^{19}\) 18β-Glycyrrhetin mono-β-D-glucuronide (GAMG) was donated by Dr. M. Kanaoka (Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University). 18x-GA, androstereone, 11β-hydroxyprogesterone and 11-ketoprogesterone were purchased from Sigma Chemical Co. (MO. U.S.A.). All other reagents were of the best commercial quality available.

**Results**

**Inhibition of 11β-HSD Activity by GA Derivatives**

Figure 3 shows the effects of GA, 18x-GA, carbenoxolone and GAMG on NADP⁺-dependent 11β-hydroxyprogesterone-oxidizing activity of rat liver microsomes. The enzyme activity was inhibited dose-dependently and potently with GA (\( I_{50} = 0.3 \mu M \)) and carbenoxolone (\( I_{50} = 1 \mu M \)) as reported by Monder et al.\(^ {14} \) Although 18x-GA and GAMG also inhibited the enzyme activity, their inhibitions (\( I_{50} = 3 \) and 10 μM) were one tenth weaker, respectively, than those of GA and carbenoxolone. GL at the concentration of 50 μM slightly inhibited the activity as reported by Monder et al.\(^ {14} \)

3-DeoxyGA and 11-deoxoGA potently inhibited 11β-HSD activity at a concentration similar to GA (Fig. 4). Their \( I_{50} \) values were 0.2 and 0.3 μM, respectively. Moreover, the inhibitory activities of their 18x-stereoisomers were one tenth of those of 18β-isomers, as was the case of 18x- and 18β-isomers of GA. 3-KetoGA and 3-epiGA were also potently inhibitory (\( I_{50} = 0.4 \mu M \)) against the enzyme activity similar to GA (data not shown).

These results suggest that 3-hydroxyl and 11-oxo groups of GA do not have an important role in the inhibition of 11β-HSD activity but the 18β-configuration of GA derivatives contributes to the inhibition.

**Inhibitory Effects of 18x- and 18β-Stereoisomers of 3-Deoxy- and 11-DeoxoGAs on NADP⁺-Dependent 3β-HSD Activity**

In the previous paper,\(^{12}\) we showed that the
18β-isomer of GA inhibited 3α-HSD activity more potently than the 18β-isomer (GA). Although both 3-deoxy- and 11-deoxo-GAs had less inhibitory effects ($I_{50} = 20 \mu M$) on the enzyme activity than GA ($I_{50} = 7 \mu M$), their 18β-isomers inhibited 3-8 fold more potently than the corresponding 18β-isomers as shown in Fig. 5. These facts indicate that the 18α-configuration of GA derivatives is important for the inhibition of 3α-HSD activity, in reverse of the case of the inhibition of 11β-HSD activity. Our previous and present results on the inhibition of 11β- and 3α-HSD activities by various GA derivatives are summarized in Table I.

Discussion
A side effect, pseudoaldosteronism, has been observed in patients given high doses of GL for prolonged periods.4,5 Recently, this side effect was found to be characteristic of the syndrome of apparent mineralocorticoid excess with 11β-HSD deficiency,20 and to be due to the potent inhibition of 11β-HSD by GA.14,21 Thus, GA potently inhibited the 11β-HSD activity of rat kidney and liver in \textit{vivo}, and orally administered GA inhibited the enzyme activity of rat kidney cortex and suppressed the plasma corticosterone level in \textit{vivo}. On the other hand, GL has little inhibitory effect on the enzyme activity, but carbeneoxalone potently inhibited it.14 In the present study, 18β-

stereoisomers of GA derivatives such as 3-deoxy- and 11-deoxoGAs showed the potent inhibition of 11β-HSD activity similar to GA. However, 18z-stereoisomers of the corresponding compounds showed weaker inhibitions (Figs. 3 and 4), indicating that the 18β-configuration of oleanane is important for the inhibition of 11β-HSD. Moreover, GA is more cytotoxic against human fibroblasts than 18z-GA,22 though GA is more effective than 18α-GA as an anti-mutagenic and anti-tumor initiating agent. Penning and Talalay proposed a good correlation between the anti-inflammatory effects of drugs and their inhibitory effects on 3α-HSD activity.23,24 In our previous report,12 18α-GA, GA, and carbeneoxalone, which have an anti-inflammatory action,11,15,26-28 potently inhibited 3α-HSD activity. Moreover, 18z-GA inhibited the enzyme activity more potently than GA, parallel to the order of the anti-inflammatory actions of these agents.11,15 For example, 18α-GA at a daily dose of 3 mg/kg p.o. exhibited a similar inhibitory effect on GA at a daily dose of 30 mg/kg p.o. on the formation of cotton pellet granuloma in mice. In the present study, 18z-isomers of 3-deoxy- and 11-deoxoGAs inhibited 3α-HSD activity more potently than the 18β-isomers (Fig. 5). Moreover, 5β-reductase activity of rat liver cytosol was also more potently inhibited by 18α-GA than by GA.11 From these findings, the planar conformation of 18α-stereostructure of oleanane, similar to the conformation of prednisolone,11 may be important for the anti-inflammatory action and the inhibition of 3α-HSD and 5β-reductase activities. Accordingly, 18β-stereoisomers such as 18z-GA are considered to be better drugs having higher anti-inflammatory properties but lower side effects.

Takahashi \textit{et al.} reported that 11-deoxoGA and 11-deoxoglycyrhetol had little inhibitory effect on 5β-reductase activity.16 It seems plausible that the 11-oxo-\textit{A}12(13) system in ring C of GA is competitive with the 3-oxo-\textit{A}4(5) system in ring A of cortical steroids at the active site of the reducing enzyme. This hypothesis is compatible with the results that both 18z- and 18β-isomers of 11-deoxoGA had the same inhibitory effects on 11β-HSD activity as the corresponding GA isomers (Fig. 4), suggesting that the 11-oxo-group in ring C of GA is not competitive with the 11-oxo-group in ring C of cortical steroids at the active site. On the other hand, 11-deoxoGA showed less inhibitory effect on 3α-HSD activity than GA (Table I). Moreover, 3-epiGA and 3-deoxyGA were weaker inhibitors against 3α-HSD than GA, and 3-deoxy-18z-GA was also weaker than 18α-GA (Table I), though 3-ketoGA had the same inhibitory efficacy as GA. These results suggest that 3β-hydroxyl and 3-ketonic groups in ring A of GA play an important role in the inhibition of 3α-HSD.

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References