

## Research Article

# The Effect of Glycyrrhetic Acid on Pharmacokinetics of Cortisone and Its Metabolite Cortisol in Rats

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The purpose of this paper is to study pharmacokinetics of cortisone (E) and its metabolite cortisol (F) in rats after administration of glycyrrhetic acid (GA) and cortisone. Healthy male SD rats were randomized to be given 20 mg/kg E or E combined with 10 mg/kg GA. Blood samples were collected at 5, 10, 20, 40, 60, 90, 120, 150, 180, and 240 min after administration. The serum concentrations of E and F were determined by HPLC and pharmacokinetic parameters were calculated using DASver2.0 software. The parameters of  $AUC_{(0-t)}$ ,  $AUC_{(0-\infty)}$ , and  $C_{max}$  for E in the group of E + GA were significantly higher than those in the group of E ( $P < 0.01$ ); the half-time ( $t_{1/2\beta}$ ) was extended compared to E ( $P < 0.05$ ) and CL/F was dropped obviously ( $P < 0.01$ ). The rise in  $AUC_{(0-t)}$ ,  $AUC_{(0-\infty)}$ , and  $C_{max}$  for cortisol in the group of E + GA was significantly compared to the group of E ( $P < 0.01$ ). CL/F was lower than E ( $P < 0.01$ ) and the half-time ( $t_{1/2\beta}$ ) was slightly extended. In this study, we find that GA restrains the metabolism of E and F and thus increases AUC,  $t_{1/2\beta}$ , and  $C_{max}$  of E and F, which may be related to its inhibition effect on  $11\beta$ -hydroxysteroid dehydrogenase ( $11\beta$ -HSD).

## 1. Introduction

Licorice is one of the most commonly used clinical drugs [1]. When glycyrrhizin is administered orally, glycyrrhetic acid is the major metabolite [2]. Glycyrrhetic acid (GA) is an important bioactive substance of licorice with a variety of pharmacological effects, such as anti-inflammatory [3], antiviral [4], antiulcer [5], and adrenal cortical hormone kind function [6]. Clinical trials clearly show that glycyrrhetic acid has a good effect on all types of dermatitis [7], purulent scar disease [8], and hair follicle infection and can cure gingivitis, esophagus inflammatory disease. It plays a physiological function in reducing lipemia and antiatherosclerotic and preventing atherosclerosis [9, 10]. Many enzymes are inhibited by glycyrrhetic acid in corticosteroids metabolic process, such as  $17\beta$ -HSD,  $11\beta$ -HSD,  $5\alpha$ -reductase, and  $5\beta$ -reductase [11–13].

Two distinct isozymes of  $11\beta$ -HSD catalyze the interconversion of hormonally active cortisol (F) and inactive

cortisone (E), which are important human glucocorticoids [14–16]. This occurs via inhibition of the enzyme by inhibiting the enzyme  $11\beta$ -hydroxysteroid dehydrogenase. Glucocorticoids have anti-inflammatory, immunosuppressive, antishock, and other pharmacological roles, with clinically important applications [17].

We develop a high performance liquid chromatography method for the simultaneous determination of cortisone and cortisol in rat serum. The pharmacokinetics of cortisone and cortisol in rats after the administration of glycyrrhetic acid and cortisone were studied.

## 2. Experimental

**2.1. Chemicals, Reagents, and Stock Solutions.** Cortisone (lot no. 0001447641, purity >98.0%) and trifluoroacetic acid (TFA) (lot no. 064K3647, purity >98.0%) were purchased from Sigma-Aldrich Company (St. Louis, MO, USA).

Cortisol and pirfenidone (both purity >98.0%) were gifts from Rockefeller University (New York, USA). All other chemicals were analytical grade and used without further purification. Purified water was prepared in house with a Milli-Q water system from Millipore (Bedford, MA, USA).

The drugs were accurately weighed ( $\pm 0.0001$  g), on an analytical balance from METTLER TOLEDO AB204-A (Zurich, Switzerland), to prepare stock solutions of individual compounds at a concentration of 1 mg/mL in methanol. All of the standard solutions were stored in the dark at 277 K for a maximum of one month. Working solutions for calibration and controls were prepared from the stock solution by dilution using methanol. The internal standard (IS) working solution (100  $\mu\text{g/mL}$ ) was prepared by diluting its stock solution with methanol. Calibration curves were prepared using blank serum spiked at concentrations of 50, 100, 250, 500, 1000, 2000, 4000, and 6000 ng/mL. Low, medium, and high quality control (QC) samples at concentrations of 100, 1000, and 4000 ng/mL were prepared in the same way as the calibration standard.

**2.2. Equipment and Conditions.** HPLC system (Agilent 1100) was equipped with quaternary pump, on-line vacuum degasser, autosampler, column compartment, diode array detector, and Agilent Chem Station Rev A.10.02. Chromatographic separation was achieved on a  $4.6 \times 150$  mm, 5  $\mu\text{m}$  particle and an Agilent Zorbax SB-C18 column was kept constant during on the run: a source of temperature of 298 K using a one-step linear gradient. Mobile phases A (acetonitrile), B (0.1% TFA), and C (water) ratio changed as follows: 0~12 min, 24~34% A, 40% B, and 36~26% C. The detector operated at 246 nm for cortisone and cortisol. The injection volume was 20.0  $\mu\text{L}$ , and the flow rate of mobile phase was 0.9 mL/min.

**2.3. Calibration Standards and Quality Control Samples.** Stock solutions of cortisone (1 mg/mL), cortisol (1 mg/mL), and IS (1 mg/mL) were separately prepared in 25 mL volumetric flasks with methanol and stored at 277 K. Working solutions for calibration and controls were prepared from the stock solution by dilution using methanol. The IS working solution (100  $\mu\text{g/mL}$ ) was prepared by diluting its stock solution with methanol. Calibration curves were prepared using blank serum spiked at concentrations of 50, 100, 250, 500, 1000, 2000, 4000, and 6000 ng/mL. Low, medium, and high quality control (QC) samples at concentrations of 100, 1000, and 4000 ng/mL were prepared in the same way as the calibration standard.

**2.4. Animals and Treatment.** Male SD rats, weighing  $300 \pm 20$  g (Certificate no. 2007-0005) were purchased from Shang-Hai SLAC Laboratory animal Co., ltd. Rats were divided into 2 groups, each group was administered with glycyrrhetic acid 10 mg/kg or vehicle (DMSO versus water = 1:9(V/V)) three times at 0, 12, and 24 h via intraperitoneal injection. Rats were anesthetized by 10% chloral hydrate before intraperitoneal injection of cortisone 20 mg/kg. Blood samples 800  $\mu\text{L}$  were directly collected into a clean tube from

the tail vein at 5, 10, 20, 40, 60, 90, 120, 150, 180, and 240 min. After centrifugation at 5000 rpm for 10 min, serum was separated and stored at 253 K until analysis.

**2.5. Sample Preparation.** The serum samples were prepared using, liquid-liquid extraction technique. A 300  $\mu\text{L}$  aliquot of the serum sample was taken in a 10 mL glass test tube, and on that 20  $\mu\text{L}$  of IS (100  $\mu\text{g/mL}$ ) was spiked and 150  $\mu\text{L}$  of sodium hydroxide solution (0.01 mol/L) was added. To this, 3 mL of extraction solvent (ethyl acetate) was added and vortex mixed on a multiple vortexer for 2.0 min, followed by centrifugation at 4000 rpm for 15 min. The supernatant was dried under nitrogen and dissolved in 200  $\mu\text{L}$  of mobile phase. A 20  $\mu\text{L}$  aliquot of the final supernatant was injected into the HPLC system for analysis.

**2.6. Method Validation.** The analytical method was validated to demonstrate the selectivity, recovery, accuracy, and precision of measurements.

Selectivity was established by the lack of interference peaks at the retention time for cortisone, cortisol, and IS.

Recovery of the method was determined at three concentration levels (100, 1000, and 4000 ng/mL) by comparing the peak area obtained from the extracted serum samples with the peak area obtained by the direct injection of the corresponding concentration spiked standard solution in the extracted blank serum.

QC samples at three concentration levels (100, 1000, and 4000 ng/mL) were analyzed to assess the accuracy and precision of the method. Intraday accuracy and precision ( $n = 6$ ) were evaluated by assays of QC samples at different times during the same day. Interday accuracy and precision were tested from the results of replicate assays of QC samples over three consecutive days. Accuracy of the method was estimated based on relative error (RE) and precision was estimated by the relative standard deviation (RSD).

**2.7. Statistical Analysis.** Data were expressed as  $\bar{x} \pm s$ . The significance of differences was assessed by unpaired  $t$ -test and one-way analysis of variance.

### 3. Results

**3.1. Method Validation.** In our study, the resolution of cortisone, cortisol, and their internal standard was satisfactory. No interference from internal materials in serum can be observed in the HPLC chromatograms. The retention time of cortisone, cortisol, and internal standard was 10.1 min, 9.3 min, and 7.6 min, respectively. The HPLC chromatograms are shown in Figure 1.

The regression equation of cortisone was  $Y_1 = 0.1991 X_1 + 0.0041$  ( $r = 0.9998$ ) and its lower limit of quantitation (LLOQ) was 0.05 mg/L ( $n = 6$ ; RSD = 3.70%); the regression equation of cortisol was  $Y_2 = 0.2262 X_2 - 0.0033$  ( $r = 0.9999$ ) and its LLOQ was 0.05 mg/L ( $n = 6$ ; RSD = 5.22%). The extractive recoveries for cortisone were (82.00  $\pm$  0.64)%, (79.68  $\pm$  2.04)%, and (81.21  $\pm$  0.98)%, respectively at different serum standard concentrations of 0.1, 1.0, and

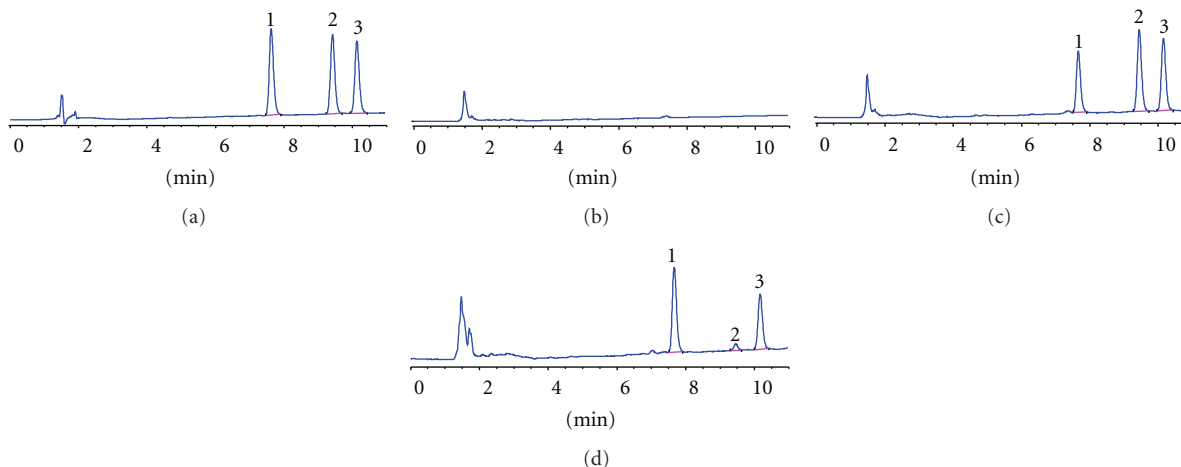


FIGURE 1: HPLC chromatograms of cortisone and cortisol. (a) Pure standard substance, (b) blank serum, (c) serum spiked with cortisone, cortisol, and internal standard and (d) sample; 1: internal standard, 2: cortisol and 3: cortisone.

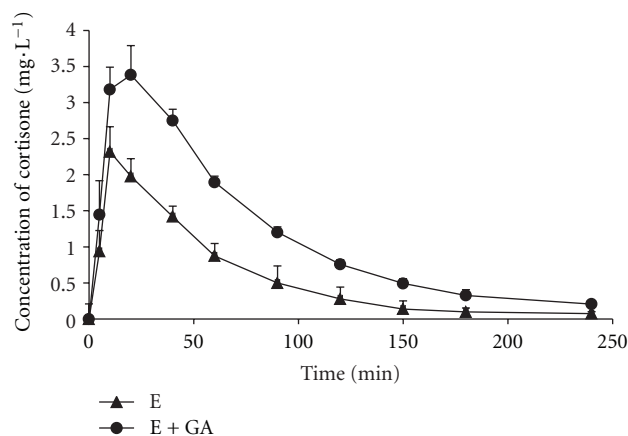


FIGURE 2: Mean concentration-time curve of cortisone in two groups  $n = 6$ .

4.0 mg/L; the extractive recoveries for cortisol at the same concentration levels were  $(78.18 \pm 1.63)\%$ ,  $(79.71 \pm 1.02)\%$ , and  $(81.47 \pm 1.18)\%$ , respectively. The intra- and interday precision (RSD) of cortisone was 4.53%, 2.34%, and 3.29% and 4.87%, 2.76%, and 3.79% at different serum standard concentrations of 0.1, 1.0, and 4.0 mg/L; the intra- and interday precision (RSD) of cortisol at the same concentration levels were 4.97%, 3.74%, and 2.67% and 4.95%, 3.56%, and 2.36%.

**3.2. Pharmacokinetic Study of Cortisone and Cortisol.** To investigate the pharmacokinetic parameters, we applied compartmental model. The mean concentration-time curves of cortisone in two groups were presented at Figure 2 and the pharmacokinetic parameters were presented at Table 1. The mean concentration-time curves of cortisol in two groups were presented in Figure 3 and the pharmacokinetic parameters were presented in Table 2.

TABLE 1: The main pharmacokinetic parameters of cortisone in two groups  $n = 6$ .

Pharmacokinetic parameters	E	E + GA
$t_{1/2\beta}/\text{min}$	$38.182 \pm 12.994$	$54.804 \pm 11.394^*$
$\text{CL}/\text{F}/\text{L} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$	$0.144 \pm 0.029$	$0.067 \pm 0.004^{**}$
$\text{AUC}_{(0-t)}/\text{mg} \cdot \text{min} \cdot \text{L}^{-1}$	$139.147 \pm 27.749$	$281.729 \pm 19.740^{**}$
$\text{AUC}_{(0-\infty)}/\text{mg} \cdot \text{min} \cdot \text{L}^{-1}$	$144.295 \pm 32.099$	$297.792 \pm 20.450^{**}$
$C_{\text{max}}/\text{mg} \cdot \text{L}^{-1}$	$2.401 \pm 0.242$	$3.516 \pm 0.223^{**}$

\*  $P < 0.05$ ; \*\*  $P < 0.01$  in comparison to E.

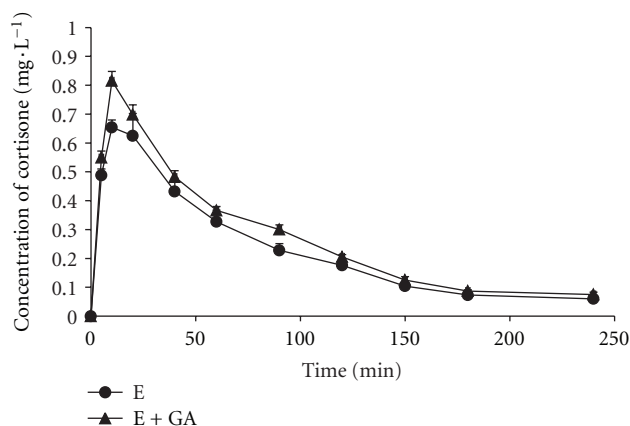


FIGURE 3: Mean concentration-time curve of cortisol in two groups  $n = 6$ .

## 4. Discussion

We studied the pharmacokinetic characteristics of E and F in rats combined using GA and E and then fitting their pharmacokinetic parameters. A two-compartment model was found meeting concentration-time curves of cortisone and cortisol in group E and E + GA.  $T_{1/2}$ ,  $\text{CL}/\text{F}$ ,  $\text{AUC}_{(0-t)}$ ,  $\text{AUC}_{(0-\infty)}$ , and  $C_{\text{max}}$  of cortisone and cortisol were affected

TABLE 2: The main pharmacokinetic parameters of cortisol in two groups  $n = 6$ .

Pharmacokinetic parameters	E	E + GA
$t_{1/2\beta}/\text{min}$	$60.859 \pm 4.065$	$62.557 \pm 5.415$
$\text{CL}/\text{F}/\text{L} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$	$0.343 \pm 0.012$	$0.284 \pm 0.011^{**}$
$\text{AUC}_{(0-t)}/\text{mg} \cdot \text{min} \cdot \text{L}^{-1}$	$53.054 \pm 2.102$	$64.416 \pm 2.776^{**}$
$\text{AUC}_{(0-\infty)}/\text{mg} \cdot \text{min} \cdot \text{L}^{-1}$	$58.411 \pm 2.038$	$70.576 \pm 2.656^{**}$
$C_{\text{max}}/\text{mg} \cdot \text{L}^{-1}$	$0.627 \pm 0.04$	$0.815 \pm 0.033^{**}$

\*  $P < 0.05$ ; \*\*  $P < 0.01$  in comparison to E.

by glycyrrhetic acid. The  $t_{1/2}$  of cortisone in group E + GA is 43% more as much as group E. AUC increased by 110%, and  $C_{\text{max}}$  by 50%, almost to 3.516 mg/L, but CL/F was significantly decreased. AUC of cortisol in group E + GA increased almost 25%,  $C_{\text{max}}$  increased by 30%, to 0.815 mg/L; however, CL/F was significantly decreased. Although  $t_{1/2}$  of both extended, no significant differences were found. Because individual differences exist in animal test, and the sample size is too small to achieve requirement of test power (power =  $1 - \beta$ ), as the result differences in parent population cannot be found out.

GA is an inhibitor of  $11\beta$ -HSD that catalyzes conversion between E and F, so the change of pharmacokinetic characteristics of E and F may be the result of affect of GA [16, 18]. Two isoforms of  $11\beta$ -HSD have been cloned [19, 20], with type I ( $11\beta$ -HSD1) as a primary reductase that converts E to F, and type II ( $11\beta$ -HSD2) as a unidirectional oxidase that converts F back to E. The effects of GA on both enzymes have been reported. Our previous research demonstrated that GA significantly increased endogenous corticosterone level and further increased its level when administrated in combination with GA [17]. In human subjects, liquorice has also been found to inhibit  $11\beta$ -dehydrogenase, as measured by a rise in the plasma half-life of F [21]. In our study, we clearly demonstrated that GA increased the AUC of E after combinatory administration, indicating that GA inhibited  $11\beta$ -HSD1, which is a primary reductase located in the liver and metabolizes E into F. However, we also witnessed the rise of F level, suggesting that GA may inhibit the further metabolism of F.

As we all know that GA can increase effective blood concentration and  $t_{1/2}$  of glucocorticoids, when combined using that it can increase the effect of anti-inflammatory, antiallergic action, antistress response, and so on [22]. It also can inhibit the hypothalamic-pituitary-adrenal-acceptor systems, decreasing and relieving some reverse effect, withdrawal symptom, and dependency, so it is often used as replacement drugs when reduce or withdraw cortical hormone [23, 24].

The result found at present study indicated that the pharmacokinetic characteristics of E and its metabolite can be changed by combined preparation of GA and E. It has significant clinical value. The wide use of GA can greatly increase compliance, but the administrate dose of GA should be noticed when using GA combined with E or acted as the alternative medicines.

Although radioimmunity is used to detect glucocorticoids usually for its high sensitivity; the result would be much higher than the actual concentration [18, 19]. Because target molecule, glucocorticoids, would cross-react with their isomers or metabolites [25]. Previous research had proved the result that conducted by ELISA and RIA assay it would have the same problem [26]. In this work, we employed an HPLC-DAD assay to detect E and F concentrations in serum. Compared to the methods mentioned above, the specificity of HPLC-DAD is much higher, so the result is much more reliable.

## Authors' Contribution

Dan Lin and Wei Sun contributed equally to this work.

## Conflict of Interests

None of the authors has any other conflict of interests related to this paper.

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