Pharmacokinetics of Intravenous Glycyrrhizin After Single and Multiple Doses in Patients with Chronic Hepatitis C Infection

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ABSTRACT

Intravenous glycyrrhizin has been used in Japan for the treatment of chronic hepatitis for >20 years, although only a few reports of its pharmacokinetic profile after multiple intravenous doses in small numbers of Japanese patients have been published. The present study compared these Japanese data against the pharmacokinetic characteristics of glycyrrhizin after single and multiple intravenous doses in 35 European patients with chronic hepatitis C infection. We administered 80, 160, or 240 mg glycyrrhizin 3 times/wk or 200 mg glycyrrhizin 6 times/wk for 4 weeks. Twenty-four–hour pharmacokinetic assessments were performed on day 1 and on or around day 14. Glycyrrhizin levels were determined by high-performance liquid chromatography. The mean (± SD) volume of distribution at steady state on day 1 in the 80-, 160-, 200-, and 240-mg groups were 67 ± 11, 62 ± 13, 54 ± 7, and 66 ± 8 mL/kg, respectively. The respective terminal elimination half-lives on day 1 were 7.7 ± 2.8, 10.1 ± 1.4, 9.0 ± 2.3, and 8.6 ± 2.1 hours. The area under the curve (AUC) increased linearly with doses ≤200 mg ($r = 0.67$; $P < 0.001$). No significant differences between day 1 and day 14 were found in any dose group, with the exception of AUC in the 200-mg group, which was significantly higher on day 14 compared with day 1 ($P = 0.03$). Comparing the European and Japanese data, the mean (± SD) AUC was 289 ± 244 μg/h per mL for the former and 402 ± 372 μg/h per mL for the latter; the half-life was 8.2 ± 2.6 versus 8.8 ± 9.0 hours; and the total clearance was 7.6 ± 3.6 versus 8.5 ± 5.7 mL/h per kg. Thus our pharmacokinetic data are comparable to those from Japan. Glycyrrhizin's pharmacokinetics are linear up to 200 mg. Drug accumulation is seen after 2 weeks of treatment with 200 mg administered 6 times/wk. *Key words:* pharmacokinetics, glycyrrhizin, hepatitis C.

INTRODUCTION

Glycyrrhizin, extracted from the roots of the plant *Glycyrrhiza glabra* (licorice), has been used as a treatment for chronic hepatitis in Japan for more than 20 years.[1] According to information from the manufacturer, Minophagen Pharmaceutical Co., Ltd. (Tokyo, Japan), tens of millions of ampoules of Stronger Neo-Minophagen C® (SNMC) containing 2 mg of glycyrrhizin per mL are used annually for this indication in Japan.

Glycyrrhizin is a conjugate of 1 molecule of glycyrrhetinic acid and 2 molecules of glucuronic acid (Figure 1). Suzuki et al[2,3] reported that intravenously administered glycyrrhizin lowered serum transaminase levels significantly in patients with chronic hepatitis. Arase et al[4] reported that in Japanese patients with chronic hepatitis C infection, normalization of alanine aminotransferase (ALT) induced by long-term glycyrrhizin treatment prevented development of hepatocellular carcinoma.

Intravenously administered glycyrrhizin is metabolized in the liver by lysosomal β-D-glucuronidase to 3-mono-glucuronide-glycyrrhetinic acid. The metabolite is excreted with bile into the intestine, where it is metabolized by bacteria into glycyrrhetinic acid, which can be reabsorbed (see Figure 1).[5]

A MEDLINE® search of the literature from January 1966 to July 1999 using the key words *glycyrrhizin(e)* and *pharmacokinetics* yielded a small number of Japanese studies involving ≤10 patients. These studies[6-9] described the pharmacokinetic profile of glycyrrhizin after multiple doses of a single dose strength in patients with acute and chronic hepatitis and cirrhosis of different causes. In these patients, total body clearance was inversely correlated with serum ALT level ($r = -0.7$; $P < 0.05$), suggesting a correlation of pharmacokinetic variables with hepatic function.

We conducted a Phase I/II clinical trial in Europe to evaluate the dose-dependent pharmacokinetics, safety, and efficacy of glycyrrhizin treatment. The safety and efficacy results have been reported elsewhere.[10] The study reported here was performed to compare the Japanese data with the pharmacokinetics of increasing doses of glycyrrhizin in European patients.
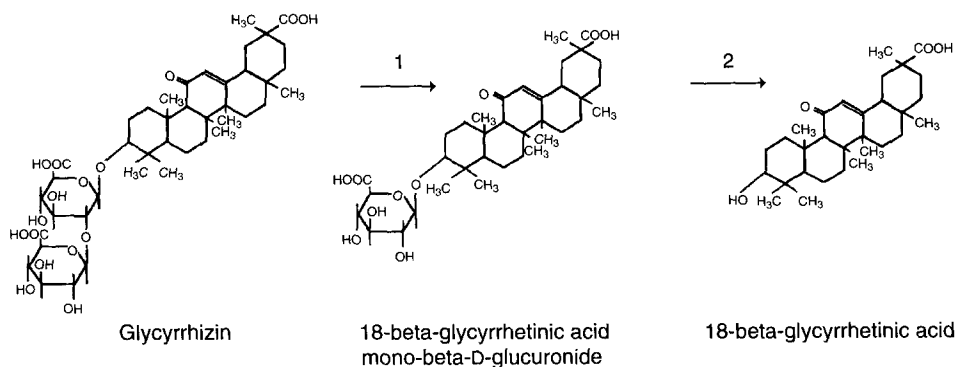

Glycyrrhizin   18-beta-glycyrrhetinic acid   18-beta-glycyrrhetinic acid
                    mono-beta-D-glucuronide

Figure 1. Metabolism of glycyrrhizin after intravenous administration: (1) in the liver, by lysosomal β-D-glucuronidase, and (2) in the intestine, by bacterial β-D-glucuronidase.

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PATIENTS AND METHODS

Only patients with chronic hepatitis C infection, with a positive hepatitis C virus RNA titer, serum ALT ≥1.5 times the upper limit of normal (ULN), and findings on liver biopsy consistent with mild to moderate liver fibrosis or cirrhosis were included. Patients were not eligible for inclusion if they had other causes of liver disease, decompensated cirrhosis (Child-Pugh score >7), or hepatocellular carcinoma.

The study was conducted according to the Declaration of Helsinki and good clinical practice guidelines. The protocol was approved by the medical ethical committee of the Erasmus University Hospital Rotterdam, and all patients gave their written informed consent.

Study Treatment

Glycyrrhizin was given as SNMC, a clear solution for intravenous use, consisting of 2 mg glycyrrhizin, 1 mg cysteine, and 20 mg glycine per mL in physiologic saline solution. Patients received 80, 160, or 240 mg intravenous glycyrrhizin 3 times/wk or 200 mg intravenous glycyrrhizin 6 times/wk for 4 weeks.

The medication administered 3 times/wk was given by drip infusion over 15 to 20 minutes in a total volume of 220 mL. The infusion line was then flushed with 25 mL sodium chloride (0.9%). The medication given 6 times/wk was administered undiluted into a peripheral vein over 3 to 5 minutes.

Pharmacokinetic Measurements

Pharmacokinetic measurements were obtained on the first day of treatment and on or around day 14. Patients were not allowed to consume food or drink (except water) after 11 PM of the night before pharmacokinetic measurements were to be taken. On the days of pharmacokinetic measurements, food was allowed 4 hours after administration of medication, and water was allowed as required. Patients remained semirecumbent from 0.5 hour before until 4 hours after receiving medication.

Blood Sampling

An indwelling cannula was placed in 1 arm for blood sampling; medication was administered in the other arm. Before sampling, ~1 mL of blood was discarded. Ethylenediaminetetraacetic acid–blood samples (7 mL) were collected at the following times on the days of pharmacokinetic measurements: before administration of medication and at 0, 5, 15, 45, 60, 90 minutes and 2, 4, 6, 8, 10, 12, 16, 18, and 24 hours after administration of medication (administration of medication was complete at time 0). Samples were stored on ice, and plasma was separated within 1 hour by centrifugation at 4°C and 3000g for 10 minutes. Plasma samples were stored at –20°C until analyzed.

High-Performance Liquid Chromatography

Plasma samples were analyzed by a validated high-performance liquid chromatographic (HPLC) method modified from Raggi et al.[11] Fifty μL propylparaben (25 mg/L internal standard solution) and 2 mL methanol were added to 500 μL plasma. After mixing and centrifugation, the supernatant was decanted into another test tube and evaporated at 40°C with flushing nitrogen. The residue was dissolved in 500 μL acetonitrile/citrate (180:320) buffer

(0.1 mol/L, pH 2.8). After vortexing and centrifugation, 20 μL of the supernatant was injected into the HPLC system. The extract was separated on a ChromSpher-5 C8 (Chrompack, Bergen op Zoom, The Netherlands) column (200 × 3 mm; 5 μm particles) with the acetonitrile/citrate buffer at a flow of 0.6 mL/min at ambient temperatures. Detection was by ultraviolet absorption at 250 nm with a diode array detector.

As validated in our laboratory, the assay was linear over a range from ≥0.5 to 150 mg/L. The limit of quantification was 0.5 mg/L. Day-to-day variations were <2% at concentrations of >30 mg/L and <5% in the lower range (~1 mg/L).

Pharmacokinetic Analysis

A weighted least-squares regression analysis, with $1/y^2$ as a weighting factor for each data point, was performed using Topfit version 2.0^{12} to analyze the plasma concentration–time data for each patient. We calculated the maximum concentration (C_{max}), total clearance (Cl_{tot}), volume of distribution at steady state (V_{ss}), area under the curve (AUC) from time zero to infinity, and terminal elimination half-life ($t_{1/2}$).

Data analysis was based on a weighted 3-compartment disposition model, which was deemed the most appropriate model based on visual inspection and minimized residuals.

Statistical Analysis

Statistical analysis was performed using Stata 5.0 software (Stata Corporation, College Station, Texas). Results are expressed as mean ± SD. Comparisons between groups were conducted using the Kruskal-Wallis test; if $P < 0.05$, the Mann-Whitney test was used to perform further pairwise comparisons between groups. Differences within groups were assessed using the Wilcoxon signed rank test. Spearman correlation coefficients were used. Significance was set at $P = 0.05$ (2-sided) for all tests.

RESULTS

The baseline characteristics of the patients are shown in Table I. The 4 groups were comparable in terms of all characteristics except baseline ALT value, which was significantly lower in the group receiving 240 mg 3 times/wk than in the group receiving 200 mg 6 times/wk ($P = 0.02$).

Figure 2 shows a chromatogram 6 hours after a single dose of 200 mg glycyrrhizin. Figure 3 shows the mean course of the measured plasma concentration on day 1 and day 14 for the group receiving 200 mg glycyrrhizin 6 times/wk. The plasma concentration of glycyrrhizin declined according to a 3-compartment model.

The second of the 2 pharmacokinetic measurements was performed on day 13 in 1 patient; day 14 in 27 patients; day 15 in 2 patients; and day 16 in 3 patients. Results for the pharmacokinetic variables are shown in Table II. A dose-response relationship was found between the C_{max} and AUC. The C_{max} increased in a log-log plot with increasing dosage (day 1, $r = 0.82$, $P < 0.001$; day 14, $r = 0.89$, $P < 0.01$), whereas there was no significant deviation from linearity. A linear increase in AUC with dose was found only for the 80-, 160-, and 200-mg doses (day 1, $r = 0.67$, $P < 0.001$; day 14, $r = 0.57$, $P < 0.002$). The mean (± SD) V_{ss} on day 1 was between 54 ± 7 and 67 ± 11 mL/kg; the mean Cl_{tot} was between 5.9 ± 2.5 and 10.3 ± 3.1 mL/h per kg; and the mean $t_{1/2}$ was be-

Table I. Baseline characteristics of the 35 patients, by glycyrrhizin dose.

	Dose, mg (No. of Patients)			
	80 (8)	160 (7)	240 (7)	200 (13)
Frequency of administration	3 times/wk	3 times/wk	3 times/wk	6 times/wk
Sex				
Male	6	5	7	11
Female	2	2	0	2
Age (y)				
Mean	47	54	43	50
Range	35–66	43–60	34–59	39–70
Weight (kg)				
Mean	71	78	79	79
Range	55–107	64–94	62–119	63–109
Cirrhosis	3	3	3	7
No cirrhosis	5	4	4	6
ALT (ULN)				
Mean	3.8	3.6	2.1*	3.9
95% CI	2.0–5.7	2.0–5.1	1.8–2.5	2.2–5.7

ALT = alanine aminotransferase; ULN = upper limit of normal.
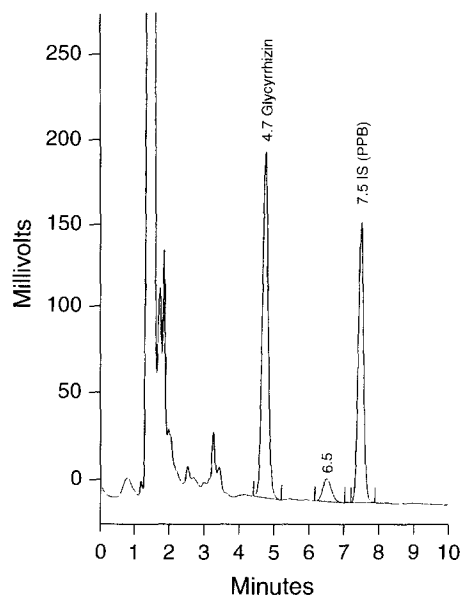*Significantly <200 mg 6 times/wk; $P = 0.02$.

Figure 2. Chromatogram of a plasma sample obtained 6 hours after a single dose of 200 mg glycyrrhizin. IS = internal standard; PPB = propylparaben.
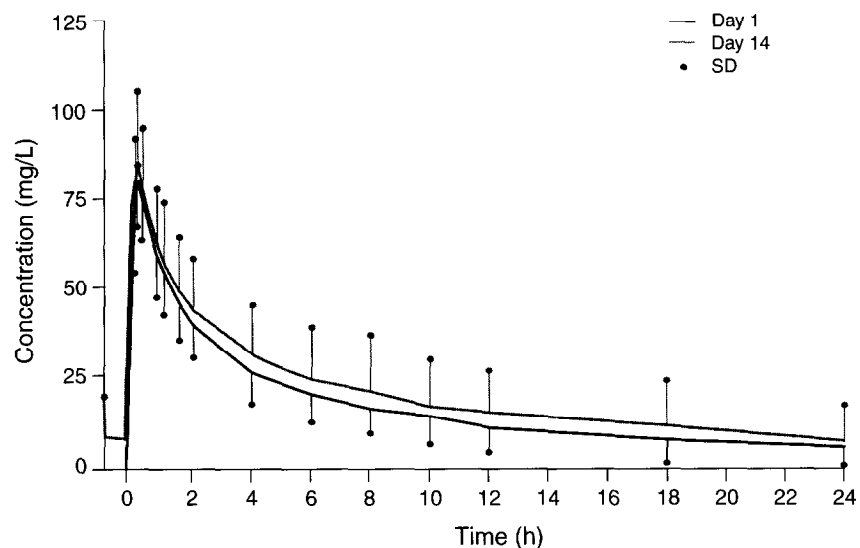
Figure 3. Mean measured plasma concentration–time profile for the group administered 200 mg glycyrrhizin on day 1 and day 14.

tween 7.7 ± 2.8 and 10.1 ± 1.4 hours. No significant difference was noted on day 1 and day 14 between dose groups, except for the significantly higher AUC in the 200-mg group on day 14 compared with day 1 (P = 0.03). All variables showed a strong correlation between day 1 and day 14.

Table III shows the Cl_{tot} and $t_{1/2}$ for cirrhotic and noncirrhotic patients on day 1 and day 14 after combining all dose groups. There were no significant differences between cirrhotic and noncirrhotic patients. No correlation could be found between ALT levels at baseline and Cl_{tot} or $t_{1/2}$.

DISCUSSION

This study reports the pharmacokinetic characteristics of increasing doses of intravenous glycyrrhizin after single and multi-

ple doses in the largest cohort of patients (35 patients) studied to date. The variation in C_{max} in the 200-mg group appeared to be substantially larger than in the other 3 groups (Figure 4). An explanation for this observation could be that the 200-mg dose was administered by manual direct intravenous injection in 3 to 5 minutes while the other 3 dosages were administered by drip infusion in 15 to 20 minutes; it is likely that the slower infusion rate caused a more equal administration of glycyrrhizin between patients than injection.

It is possible that different rates of infusion might affect the dose linearity and number of compartments. However, in our study, the AUC increased linearly with dose between 80 to 200 mg, although the 80- and 160-mg doses were administered over 15 to 20 minutes, and the 200-mg dose was

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Table II. Pharmacokinetic results, by dosage group, on days 1 and 14.

	80 mg 3 times/wk		160 mg 3 times/wk		240 mg 3 times/wk		200 mg 6 times/wk	
	Day 1 (n = 8)	Day 14 (n = 7)	Day 1 (n = 7)	Day 14 (n = 7)	Day 1 (n = 7)	Day 14 (n = 7)	Day 1 (n = 11)	Day 14 (n = 12)
C_{max} (mg/L)	42 ± 16	31 ± 4	70 ± 14	72 ± 11	102 ± 7	106 ± 16	112 ± 39	116 ± 40
V_{ss} (mL/kg)	67 ± 11	66 ± 10	62 ± 13	57 ± 15	66 ± 8	63 ± 10	54 ± 7	53 ± 14
AUC (μg/h/mL)	138 ± 76	112 ± 37	415 ± 156	466 ± 232	319 ± 66	345 ± 99	468 ± 210	574 ± 389
Cl_{tot} (mL/h/kg)	9.9 ± 3.3	10.8 ± 2.9	5.9 ± 2.5	5.7 ± 2.7	10.3 ± 3.1	9.8 ± 3.2	6.0 ± 2.6	5.5 ± 2.6
$t_{1/2}$ (h)	7.7 ± 2.8	6.2 ± 2.7	10.1 ± 1.4	10.2 ± 1.6	8.6 ± 2.1	6.6 ± 2.0	9.0 ± 2.3	9.1 ± 2.2

Values are expressed as mean ± SD.
C_{max} = maximum concentration; V_{ss} = volume of distribution at steady state; AUC = area under the curve; Cl_{tot} = total clearance; $t_{1/2}$ = half-life.

Table III. Total clearance (Cl_{tot}) and half-life $(t_{1/2})$ for patients with chronic hepatitis and cirrhosis on days 1 and 14.

	Chronic Hepatitis		Cirrhosis	
	Day 1 (n = 19)	Day 14 (n = 17)	Day 1 (n = 14)	Day 14 (n = 16)
Cl_{tot} (mL/h/kg)	8.3 ± 3.0	8.1 ± 3.2	7.2 ± 4.0	7.1 ± 4.0
$t_{1/2}$ (h)	8.2 ± 2.2	7.7 ± 2.5	9.7 ± 2.3	8.7 ± 2.7

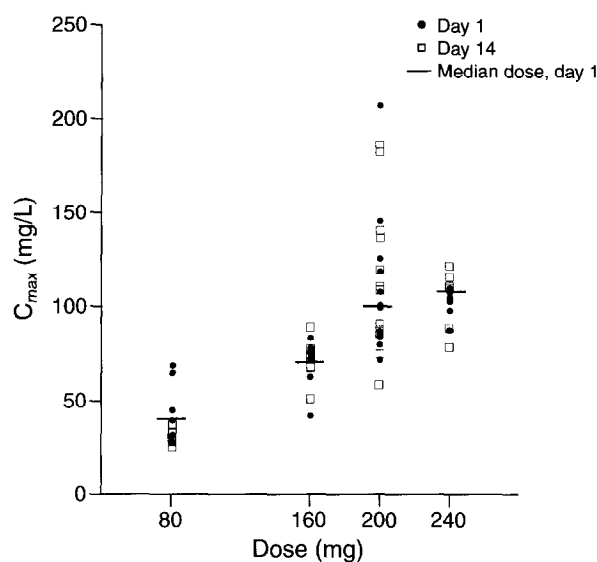Results are expressed as mean ± SD.

Figure 4. Calculated maximum concentration (C_{max}) per patient per dose on day 1 and day 14.

given over 3 to 5 minutes. The pharmaco-kinetic data after both administration rates fitted best according to a 3-compartment model. So the difference between administration over 3 to 5 minutes or 15 to 20 minutes does not appear to affect dose linearity or compartmentalization.

With glycyrrhizin's half-life of ~9 hours, a dosing interval of 24 hours might lead to some accumulation. Indeed, the AUC for the group receiving 200 mg 6 times/wk was significantly higher at day 14 compared with day 1; after 14 days of treatment, the mean (± SD) glycyrrhizin concentration before administration of medication was 7.8 ± 11.0 mg/L.

Our pharmacokinetic data are based on samples taken between 0 minutes and 24 hours after administration of medication. We observed a 3-compartment distribu-

tion over the entire period. Tanaka et al[6] investigated the pharmacokinetic profile of multiple doses of intravenously administered glycyrrhizin 120 mg in 8 patients with chronic hepatitis of unreported cause, and Yamamura et al[7] investigated the same regimen in 4 patients with acute hepatitis and 6 patients with cirrhosis (5 of 6 cases were caused by chronic hepatitis C infection). In these studies, samples were taken between 2 and 10 hours after drug administration. Over this 8-hour period, the investigators observed a monoexponential decline in glycyrrhizin, as in our study.

Combining the pharmacokinetic data on the 8 patients from Tanaka et al[6] and the 10 patients from Yamamura et al[7] yields a mean (± SD) $t_{1/2}$ of 8.8 ± 9.0 hours, a Cl_{tot} of 8.5 ± 5.7 mL/h per kg, and an AUC of 402 ± 372 µg/h per mL. These data obtained after multiple doses of glycyrrhizin are comparable to those obtained in our study (Table IV).

The $t_{1/2}$ of glycyrrhizin in 3 healthy volunteers has been reported as 3.5 hours.[13,14] Yamamura et al[13] observed an increase in $t_{1/2}$ and a decrease in Cl_{tot} among cirrhotic patients compared with noncirrhotic patients. We found no significant differences between cirrhotic and noncirrhotic patients (Table III). Cl_{tot} and $t_{1/2}$ are pharmacokinetic parameters that depend on physiologic variables. Therefore it seems logical that a decrease in hepatic function would lead to a decrease in Cl_{tot} and an increase in $t_{1/2}$. We did not observe such a relationship in the present study, probably because patients with severe liver disease were excluded. Mean ALT levels before the initiation of treatment in the Japanese patients were ~300 IU/L, much higher than our baseline ALT levels of ~3.5 times the ULN, or 150 IU/L.

Comparisons of pharmacokinetic data between centers should be interpreted cautiously. Variations in population, degree of disease, dose rate and regimen, sampling frequency, and duration of observation are all capable of influencing study results. Given these limitations, we conclude that our data corroborate and strengthen those from the smaller studies.[6,7]

The first phase of the 3-compartment model can be explained predominantly by the distribution of glycyrrhizin. The V_{ss} was ~4.5 L/patient, which means that glycyrrhizin was confined mainly to the vascular compartment. Glycyrrhizin is not taken up in blood cells.[13] The second phase can be explained by elimination, predominantly through the metabolism of glycyrrhizin to 3-mono-glucuronide-glycyrrhetinic acid in the liver by lysosomal β-D-glucuronidase.[5] The third phase

Table IV. Japanese and European pharmacokinetic data after multiple doses.

	Japan (N = 18)	Europe (N = 33)
AUC for 120 mg (µg/h/mL)	402 ± 372	289 ± 244
Cl_{tot} (mL/h/kg)	8.5 ± 5.7	7.6 ± 3.6
$t_{1/2}$ (h)	8.8 ± 9.0	8.2 ± 2.6

Results are expressed as mean ± SD.
AUC = area under the curve; Cl_{tot} = total clearance; $t_{1/2}$ = half-life.

can be explained by an enterohepatic cycle of glycyrrhizin, which would extend the elimination phase.[15,16]

CONCLUSIONS

Glycyrrhizin exhibits linear pharmacokinetics up to 200 mg; steady-state kinetics are attained after 2 weeks of 200 mg administered 6 times/wk. Our pharmacokinetic data are comparable to the Japanese findings, although we did not find a correlation between hepatic function and pharmacokinetics. This difference may be explained by our patients having milder liver disease.

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