Received: 11 March 2021 | Revised: 11 March 2021 | Accepted: 1 April 2021

DOI: 10.1111/cts.13063

ARTICLE

Safety, tolerability, and pharmacokinetics of oral baicalein tablets in healthy Chinese subjects: A single-center, randomized, double-blind, placebo-controlled multiple-ascending-dose study

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Funding information
No funding was received for this work.

Abstract

Baicalein is a biologically important flavonoid in extracted from the Scutellaria baicalensis Georgi, which can effectively inhibit the influenza virus. This study aimed to analyze the safety and pharmacokinetic (PK) characteristics of baicalein tablets in healthy Chinese subjects and provide more information for phase II clinical trials. In this multiple-ascending-dose placebo-controlled trial, 36 healthy subjects were randomized to receive 200, 400, and 600 mg of baicalein tablet or placebo once daily on day 1 and day 10, 3 times daily on days 4–9. All groups were intended to produce safety and tolerability outcomes (lowest dose first). Blood and urine samples were collected from subjects in the 600 mg group for baicalein PK analysis. Our study had shown that Baicalein tablet was generally safe and well-tolerated. All adverse events were mild and resolved without any intervention except one case of fever reported in the 600 mg group, which was considered as moderate but not related with baicalein as judged by the investigator. Oral baicalein tablets were rapidly absorbed with peak plasma levels being reached within 2 h after multiple administration. The highest urinary excretion of baicalein and its metabolites peaked in 2 h, followed by 12 h, with a double peak trend.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Many studies have shown that baicalin has an anti-influenza effect in cell and animal experiments. The primary mechanism of action is that baicalein has a strong inhibitory effect on the sialidase of the influenza virus.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study aimed to analyze the safety and pharmacokinetic (PK) characteristics of baicalein tablets in healthy Chinese subjects and provide more information for phase II clinical trials.

Trial registration numbers: CTR20140263 and CTR20140267.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Our study results have shown that baicalein tablets were administered multiple times within the studied dose range were safe and well-tolerated in healthy Chinese subjects with no serious or severe adverse effects. The highest urinary excretion of baicalein and its metabolites peaked in 2 h, followed by 12 h, with a double peak trend. Oral baicalein tablets were rapidly absorbed with peak plasma levels reached within 2 h after multiple administration.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Our study addresses the safety outcomes of baicalein tablets and emphasizes the PKs of baicalein, which provides a better understanding and a scientific basis of the clinical application of baicalein for further evaluation.

INTRODUCTION

Baicalein is one of the main components in the dried roots of Scutellaria baicalensis and a biologically important flavonoid. Recent studies have shown that baicalein has antiviral, antibacterial, anti-inflammatory, antitumor, and antioxidant functions.[1,2] In vitro tests have shown that the extract from Scutellaria baicalensis can significantly suppress influenza virus, respiratory syncytial virus, and herpes simplex virus type I. It also has certain inhibitory effects on adenovirus type 3, measles virus, and coxsackievirus B3.[3,4] In vivo studies confirmed that the extract from Scutellaria baicalensis can effectively alleviate symptoms of viral pneumonia caused by influenza virus. Some literatures have confirmed that baicalein and baicalin are the main antiviral components of Scutellaria baicalensis. The main mechanism of action is that baicalein has a strong inhibitory effect on sialidase of influenza virus.[5] In addition, the immunomodulatory function of baicalein also plays a role in inhibiting the virus.

Our previous single-ascending-dose study showed that baicalein tablets were safe and well-tolerated in the range of 100–800 mg in healthy Chinese subjects. Baicalein could be metabolized rapidly and extensively in vivo, producing a variety of metabolites, including baicalein-6-O-glucuronide (6-BG), baicalein-6-O-sulfate (6-BS), baicalein-7-O-glucuronide (7-BG), baicalein-7-O-sulfate (7-BS), baicalein-O-diglucuronide (BGG), baicalein-O-glucose-O-glucuronide (BGGlu), and methyl-O-baicalein-6-O-glucuronide (MeBG) (Figure S1).[6] After being metabolized in the body, baicalein was mainly excreted in the form of metabolites. The aims of this phase I, randomized, double-blind, placebo-controlled dose-escalation study was to evaluate the safety, tolerability, and pharmacokinetic (PK) profile of multiple-dose of baicalein tablets in healthy Chinese subjects and provide a basis for the phase II clinical trial of baicalein tablets against influenza virus.

METHODS

Research design

This study was a phase I single-center, randomized, double-blind, placebo-controlled, multiple-ascending-dose study conducted in Affiliated Hospital of Academy of Military Medical Sciences from September 2013 to January 2014 (Figure 1). Randomization codes were generated by SAS software (version 9.2).

The study included 3 dose groups of 200, 400, and 600 mg. 12 subjects were enrolled in each dose group, and randomized to receive the test drug (10 subjects) or placebo (2 subjects). A higher-dose group was not initiated unless the safety evaluation from the lower-dose cohort were acceptable. During the study, the subjects were required to be admitted to the study site 1 day before drug administration (day −1). After overnight fasting (≥10 h), the subjects were administrated with the first dose on day 1 around 8:00 a.m. Then the subjects received the test drug or placebo orally every 8 h from day 4 to day 9 for 6 consecutive days. The last dose was given on day 10 around 8:00 a.m. after overnight fasting (≥10 h). All subjects were given a standardized meal 4 h after dosing. All the subjects were followed up for 2 weeks.

According to the results of the single-dose PK study of baicalein ranged from 200 to 800 mg, that baicalein exhibited a nonlinear PK profile with less than dose proportional increases in exposure. The exposure of baicalein started to reach a plateau at the dose level of 600 mg. This may be due to the saturation of baicalein absorption. Therefore, in this multiple-dose study, blood and urine samples were collected from subjects in the 600 mg group for baicalein PK analysis.

The written informed consent was obtained before each subject's participation in this trial. The protocol was reviewed and approved by the Ethical Committee of Affiliated Hospital of Academy of Military Medical Sciences.

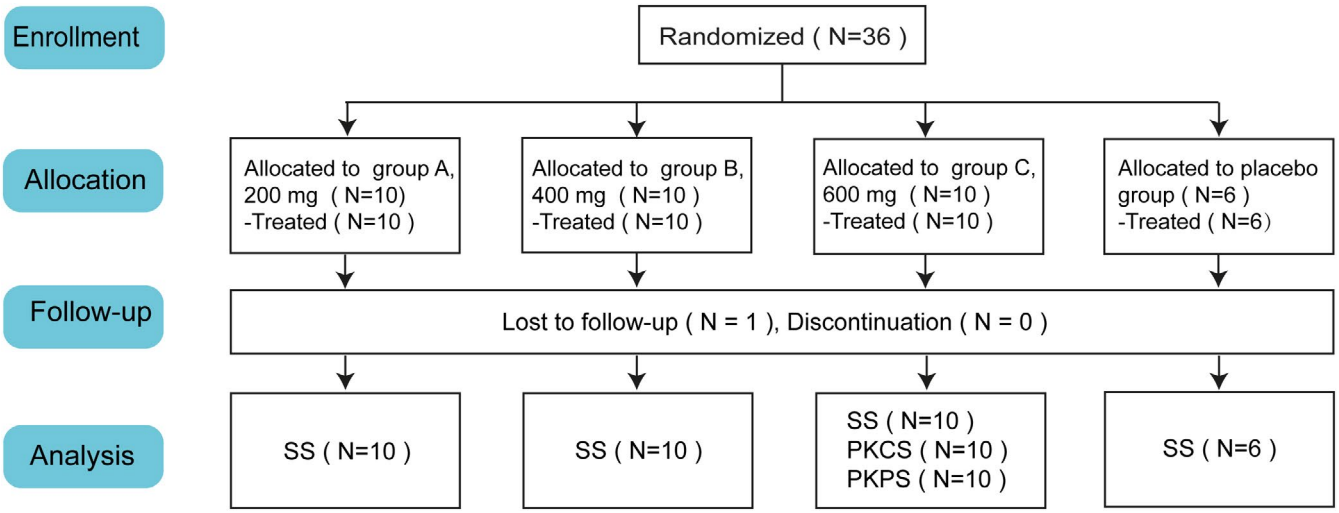

FIGURE 1 Subjects disposition. Safety set (SS): all the subjects enrolled in the study were included in the safety set. Pharmacokinetic concentration set (PKCS): all subjects with at least one drug concentration result. Pharmacokinetic parameter set (PKPS): all subjects that had at least one viable PK parameter

Subjects

Healthy subjects agreed to participate in this clinical study and signed informed consent with age between 18 and 40 years, body mass index (BMI) within 19.00–24.00 kg/m^2 (inclusive). Baseline assessments included medical history, physical examination, height, weight, BMI, vital signs, clinical laboratory tests, and 12-lead electrocardiograms (ECGs). Eligible subjects were healthy based on a medical evaluation within 1 week before the trial, including medical history, physical examination, height, weight, BMI, vital signs, clinical laboratory tests, and 12-lead ECGs. Eligible subjects also needed to have normal communication ability and follow the relevant regulations. Women of childbearing age must have a negative pregnancy test prior to study entry.

Key exclusion criteria were: a history of drug allergy; a plan for pregnancy; use of other drugs within 21 days of the first dose of the test drug; participation in any drug trial in the past 4 months; a history of drug dependence (except smoking) or drug abuse in the past 2 years; a history of regular alcohol consumption; smoking history with more than 5 cigarettes daily; a blood loss or donation up to 200 ml within 3 months before the trial; positivity for hepatitis B, hepatitis C, HIV, or syphilis antibodies; and any clinically significant abnormalities (as judged by the investigator).

Test drugs

In this study, both baicalein tablets and placebo tablets were provided by CSPC ZhongQi pharmaceutical technology Co. Ltd in accordance with Good Manufacturing Practice. The test drug was 500 mg/tablets, which contained baicalein 100 mg/tablets. The placebo tablet was made with the same excipients without the active ingredient.

Safety assessment

All the subjects were included in the safety analysis. Baseline assessments were performed before the administration of the drug. Subjects were monitored carefully throughout the entire study. Safety assessments included adverse events (AEs), physical examination, clinical laboratory examination, vital signs, and 12-lead ECG examination. Laboratory tests included complete blood count, liver function, kidney function, blood lipid test, and urinalysis.

Pharmacokinetic analysis

As mentioned in previous literature,[7–9] a liquid chromatography-tandem mass spectrometry analysis method was used to identify and quantify baicalein and its seven metabolites (6-BG, 6-BS, 7-BG, 7-BS, BGG, BGGlu, and MeBG) in human plasma and urine. The samples were quantified by multiple reaction monitoring. The ion reactions used for quantitative analysis were as follows: *m/z* 271.0 → *m/z* 122.8 (baicalein), *m/z* 447.1 → *m/z* 271.1 (6-BG and 7-BG), *m/z* 351.0 → *m/z* 271.0 (6-BS and 7-BS), *m/z* 623.2 → *m/z* 271.0 (BGG), *m/z* 461.1 → *m/z* 284.9 (MeBG), and *m/z* 609.1 → *m/z* 271.1 (BGGlu).

Blood samples was collected at the following time points: predose and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 60, and 72 h postdose on days 1 and 10. Two additional samples were collected on days 8 and 9 (predose).

Urine samples were collected at the following timepoints: predose and 0–2, 2–4, 4–6, 6–8, 8–12, 12–24, 24–36, 36–48, 48–60, and 60–72 h postdose on days 1 and 10.

The PKs of baicalein and its metabolites were profiled by the maximum drug plasma concentration after dosing (C_{max}), time to C_{max} (T_{max}), area under the concentration-time curve from time zero to the last measurable concentration (AUC_{0-t}), area under the concentration-time curve from time zero to infinity ($AUC_{0-\infty}$), terminal phase half-life (HL_Lambda_z), apparent total plasma clearance, apparent total plasma clearance in steady-state, apparent total volume of distribution, minimum drug plasma concentration after dosing, average steady-state concentration, steady-state area between a dosing interval ($AUC_{0-\tau,ss}$), and degree of fluctuation.

Statistical analysis

Safety was assessed by AEs and changes in laboratory test results, vital signs, and ECGs in all subjects enrolled in the study (safety set). Descriptive statistical analysis is the primary method for safety evaluation. Categorical variables were described by percentage or composition ratio, and continuous variables were described by the mean and SD, or by median (minimum, maximum) as appropriate.

Plasma drug concentration-time profile was characterized using the PK concentration set, which consisted of subjects with at least one drug concentration result. PK parameters were calculated with the PK parameter set, which included subjects that had at least one viable PK parameter.

The AUC values were calculated by the linear-up log-down method. These PK parameters were derived by non-compartmental analysis and summarized as the mean (SD) unless otherwise specified. The accumulation ratio of baicalein were calculated by $AUC_{0-\tau,ss}/AUC_{0-\tau,1}$ and $C_{max,ss}/C_{max,1}$. Steady-state conditions were evaluated by analysis of mean trough (predose) concentrations on days 8 through 10. The main PK parameters (T_{max}, C_{max}, and AUC) were compared between genders by rank sum test or *t*-test depending on the distribution of data.

All statistical analyses were performed using SAS version 9.2 (SAS Institute), except for PK data, which were analyzed using Phoenix WinNonlin version 6.1.2 (Pharsight).

RESULTS

Subjects

Thirty-six healthy subjects were enrolled in the multiple-dose study, including 10 subjects in the 200 mg dose group, 10 subjects in the 400 mg dose group, 10 subjects in the 600 mg dose group, and 6 subjects in the placebo group. Among the 36 healthy subjects, one subject in 400 mg group dropped off due to loss of follow-up at the end of the study, and this subject was still included in the safety and PK analysis. The demographic characteristics of all enrolled subjects are presented in Table 1.

Safety

Twenty-five AEs were reported by 18 subjects after the administration of baicalein tablets or placebo. In the experimental group, 17 subjects (56.67%) reported 24 AEs. One subject (16.67%) in the placebo group reported one AE. The most common AEs were proteinuria ($n = 10$), high triglyceride ($n = 3$), and urine leukocyte positive ($n = 2$). Individual AEs are shown in Table 2. All AEs were mild to moderate in severity, requiring no medical intervention, except that one subject in the 600 mg group had a moderate fever and received indomethacin suppository. All the AEs resolved within 1 month during the study.

Eight drug-related AEs were seen during the trial period, including proteinuria ($n = 2$, 1 in 400 mg group and 1 in 600 mg group), elevated hs-CRP level ($n = 1$, 200 mg group), high triglycerides ($n = 3$, 1 in 400 mg group and 2 in 600 mg group), elevated alanine aminotransferase level ($n = 1$, 400 mg group), and elevated aspartate aminotransferase level ($n = 1$, 400 mg group). All drug-related AEs were mild and resolved during the study without further treatment.

TABLE 1 Summary of demographics and baseline characteristics

	Placebo ($N = 6$)	200 mg ($N = 10$)	400 mg ($N = 10$)	600 mg ($N = 10$)
Female, *N* (%)	3 (50%)	5 (50%)	5 (50%)	5 (50%)
Age (year)	25.00 (3.41)	25.00 (2.83)	25.30 (2.21)	25.50 (3.10)
Height (cm)	168.25 (5.80)	162.70 (9.01)	165.35 (4.20)	166.75 (6.58)
Weight (kg)	63.33 (8.12)	58.49 (7.87)	58.39 (4.05)	61.53 (7.98)
BMI (kg/m^2)	22.25 (1.78)	21.99 (1.61)	21.30 (1.23)	22.02 (1.85)

Note: Data were described by mean (SD).

Abbreviations: BMI, body mass index; *N*, number of patients; SD, standard deviation.

TABLE 2 AEs in healthy subjects included in the multiple-dose safety analysis

Type of AEs (*n*)	Placebo (*N* = 6)	200 mg (*N* = 10)	400 mg (*N* = 10)	600 mg (*N* = 10)	Total
Any AEs[a]	1	3	9	12	25
Elevated ALT level	0	0	1	0	1
Elevated AST level	0	0	1	0	1
Elevated serum creatinine	0	1	0	0	1
Elevated level of total bilirubin	0	0	1	0	1
Elevated TSBA level	0	0	0	1	1
Elevated hs-CRP level	0	1	0	0	1
High triglycerides	0	0	1	2	3
Urine leukocyte positive	0	0	0	2	2
Proteinuria	1	1	3	5	10
Microscopic hematuria	0	0	1	0	1
High urine specific gravity	0	0	0	1	1
Elevated urobilinogen level	0	0	1	0	1
Fever	0	0	0	1	1

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; hs-CRP, high-sensitivity C-reactive protein; *n*, number of adverse events; *N*, number of patients; TSBA, total serum bile acid.

[a]Includes subjects who reported at least one adverse event.

The frequency of drug-related AEs was not associated with the increased dosage.

Plasma pharmacokinetics

Oral baicalein tablets were rapidly absorbed with peak plasma levels being reached with 2 h after multiple administration. Based on the mean plasma trough concentration-time profiles (Figure 2b), steady-state concentration of baicalein was achieved after 6 days of multiple dosing, and the mean C_{avg} and $AUC_{0-\tau,ss}$ of baicalein were 633.64 (290.36) ng/ml and 5069.16 (2322.87) h ng/ml for 600 mg. The mean plasma concentration of baicalein was higher after repeated dosing compared to the first day of administration, as shown in Figure 2a.

The PK parameters of baicalein and three major metabolites (7-BG, 7-BS, and BGG) derived by the noncompartmental analysis are shown in Table 3. PK parameters of baicalein metabolites (6-BG, 6-BS, MeBG, and BGGlu) are shown in Table S1. Compared to the first administration, the HL_Lambda_z and T_{max} of baicalein and its main metabolites after repeated administrations were basically the same, whereas the in vivo exposure of C_{max} and AUC_{0-t} increased with the increase of administration days. The mean accumulation ratio for AUC of baicalein ($R_{AUC0-\tau}$) was 2.90 (1.64) and mean accumulation ratio for C_{max} of baicalein was 2.91 (2.58). There was a moderate accumulation of baicalein following multiple doses according to the critical values for moderate accumulation ($2 \leq R_{ac} < 5$) from Zheng et al.[10]

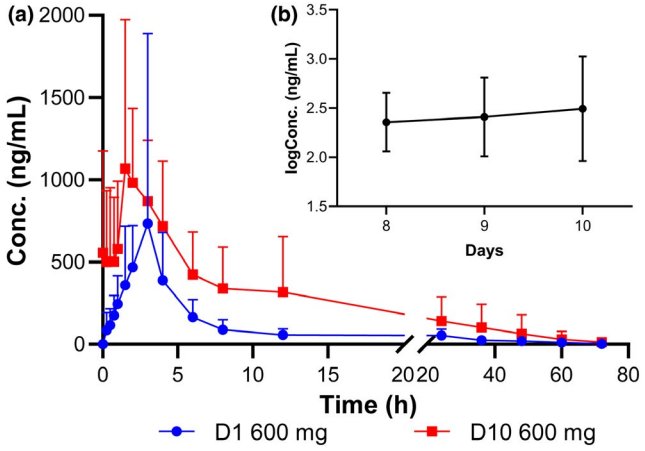

FIGURE 2 (a) The mean plasma concentration (Conc.) versus time profiles of baicalein ($N = 10$). (b) Trough concentrations of baicalein before a dose 6 (day 8), 7 (day 9), and 8 (day 10) Within each dose group, the mean value at each time point was shown

The main PK parameters T_{max}, C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were analyzed for gender difference after single and multiple administration. The p values for AUC_{0-t} and $AUC_{0-\infty}$ of baicalein were significantly different ($p < 0.05$) after the first administration, whereas T_{max} and C_{max} were not significantly different ($p > 0.05$). After repeated administrations, the results were consistent with T_{max} and C_{max} having no significant difference between genders, and AUC_{0-t} and $AUC_{0-\infty}$ significantly differed between female and male subjects. The AUC_{0-t} and $AUC_{0-\infty}$ obtained from

TABLE 3 Pharmacokinetic parameters of baicalein and its major metabolites (7-BG, 7-BS, and BGG)

Parameter	Baicalein	7-BG	7-BS	BGG
Day 1				
HL_Lambda_z (h)	14.91 (9.91)	12.48 (6.61)	13.54 (8.75)	15.46 (6.51)
T_{max} (h)	2.70 (1.06)	2.20 (1.14)	2.55 (1.12)	6.90 (6.19)
C_{max} (ng/ml)	845.20 (1122.98)	505.15 (384.47)	6460.00 (2796.55)	2061.90 (763.49)
AUC_{0-t} (h ng/ml)	4380.65 (3225.68)	3514.33 (2511.07)	39703.46 (18314.01)	32171.14 (12461.07)
$AUC_{0-\infty}$ (h ng/ml)	4494.88 (3184.48)	3712.57 (2584.56)	41673.87 (20244.48)	38948.77 (14872.48)
CL/F (L/h)	182.95 (92.74)	NA	NA	NA
V_z/F (L)	4332.47 (3651.88)	NA	NA	NA
Day 10				
HL_Lambda_z (h)	11.29 (4.20)	9.35 (3.52)	10.45 (3.61)	11.76 (5.05)
T_{max} (h)	1.71 (1.04)	2.06 (1.28)	2.60 (1.41)	2.71 (0.82)
$C_{max,ss}$ (ng/ml)	1322.50 (901.79)	1508.45 (895.02)	15080.00 (17857.99)	3716.00 (699.57)
AUC_{0-t} (h ng/ml)	12384.96 (9226.29)	10071.76 (5953.94)	111900.83 (106623.74)	58787.01 (17712.77)
$AUC_{0-\infty}$ (h ng/ml)	12580.06 (9283.08)	10175.14 (6045.02)	113286.25 (106758.07)	61862.14 (17927.86)
C_{min} (ng/ml)	285.11 (260.41)	240.20 (117.03)	3399.00 (2999.36)	1626.50 (693.85)
C_{avg} (ng/ml)	633.64 (290.36)	660.05 (315.22)	7395.08 (7383.55)	2630.05 (547.60)
DF (%)	168.43 (123.23)	181.43 (64.64)	142.74 (39.18)	81.82 (22.69)
$AUC_{0-\tau,ss}$ (h ng/ml)	5069.16 (2322.87)	5280.44 (2521.79)	59160.67 (59068.43)	21040.38 (4380.79)
CL_{ss}/F (L/h)	138.81 (55.09)	NA	NA	NA
V_z/F (L)	2499.71 (1857.02)	NA	NA	NA

Abbreviations: 7-BG, baicalein-7-O-glucuronide; 7-BS, baicalein-7-O-sulfate; $AUC_{0-\infty}$, area under the concentration-time curve from time zero to infinity; AUC_{0-t}, area under the concentration-time curve from time zero to the last measurable concentration; $AUC_{0-\tau,ss}$, steady-state area between a dosing interval; BGG, baicalein-O-diglucuronide; Cavg, average steady-state concentration; CL/F, apparent total plasma clearance; CL_{ss}/F, apparent total plasma clearance in steady-state; C_{max}, maximum drug plasma concentration after dosing; $C_{max,ss}$, maximum drug plasma concentration in steady-state; C_{min}, minimum drug plasma concentration after dosing; DF, degree of fluctuation; HL_Lambda_z, terminal phase half-life; T_{max}, time to C_{max}; V_z/F, apparent total volume of distribution.

female subjects were higher than those of male subjects. The detailed results are shown in Table 4. However, in regard to the limited sample size, the gender effect remains to be further studied.

Urine pharmacokinetics

The analysis of the cumulative excretion rate of baicalein and its metabolites in the urine showed that the total cumulative excretion rate over 72 h after the last dose was 24.75%. The total urinary excretion of baicalein and its metabolites first peaked around 2–6 h and reached the second peak around 12–24 h after a single dose (day 1). Analysis of the cumulative urinary excretion rate by time showed that the peak value of urinary excretion rate after multiple administration of 600 mg was 5 times higher than that of a single dose. In addition, the highest excretion rate was reached in the interval of 0–2 h and 8–12 h after the last dose (day 10), suggesting a bimodal distribution of urinary excretion, which was consistent with the previous results of the single-dose study. The subject's average urine excretion rates at 72 h after the first and the last dose are shown in Table 5.

DISCUSSION

Our previous study had shown that a single-dose of oral baicalein tablets within the dosage range of 100–800 mg was safe and tolerable in healthy subjects, and no serious or severe adverse effects were observed. The present study was designed to investigate the safety, tolerability, and PKs of baicalein after multiple-dose administration in healthy Chinese subjects.

Based on the safety and PK results, we selected 200, 400, and 600 mg for the multiple-dose study to acquire more information to support subsequent study in patients. The duration of the whole study was 12 days, including an initial dose (once a day) on day 1 and repeated administration (3 times a day) on days 4–9, then the final dose (once a day) on day 10.

Baicalein undergoes rapid and extensive metabolism in the body, producing a variety of metabolites. Among the 7 metabolites, 7-BS and BGG showed very high concentration in plasma, which were far higher than baicalein. After being metabolized in the body, baicalein is mainly excreted in the form of metabolites. These results were consistent with our previous single-dose study and the multiple-dose study conducted by Pang et al.[11] Baicalein and baicalin (7-BG) have been proved to have antiviral activity in the literature.[12]

TABLE 4 Comparison of the main pharmacokinetic parameters of baicalein between genders

Gender		T_{max}	Ln C_{max}	Ln AUC_{0-t}	Ln $AUC_{0-\infty}$
Single administration					
Male	$N = 5$	2.00 (1.00, 4.00)	5.84 (0.58)	7.79 (0.28)	7.83 (0.27)
Female	$N = 5$	3.00 (2.00, 4.00)	6.77 (0.91)	8.60 (0.60)	8.64 (0.56)
p value		0.4432	0.0905	0.0255	0.0182
Multiple administration					
Male	$N = 5$	2.02 (1.50, 3.02)	6.93 (0.72)	8.87 (0.30)	8.89 (0.29)
Female	$N = 5$	1.50 (0.00, 2.02)	7.11 (0.49)	9.60 (0.63)	9.62 (0.62)
p		0.1653	0.6665	0.0460	0.0439

Note: Data for T_{max} were described by median (minimum, maximum). Data for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were described by mean (SD).

Abbreviations: $AUC_{0-\infty}$, area under the concentration-time curve from time zero to infinity; AUC_{0-t}, area under the concentration-time curve from time zero to the last measurable concentration; C_{max}, maximum drug plasma concentration after dosing; Ln, natural logarithm; *N*, number of patients; SD, Standard deviation; T_{max}, time to C_{max}.

TABLE 5 Average cumulative excretion rate of urine after multiple administrations of 600 mg baicalein

Compound	Day 1 Recovered%	Day 10 Recovered %
Baicalein	0.57 (0.41)	2.91 (1.85)
6-BG	0.77 (0.86)	2.66 (1.21)
6-BS	0.18 (0.21)	0.69 (0.40)
7-BG	1.87 (2.20)	6.24 (4.12)
7-BS	2.78 (3.86)	9.53 (4.99)
BGG	3.43 (2.73)	10.94 (4.37)
BGGlu	1.43 (1.24)	4.41 (2.42)
MeBG	0.62 (0.63)	3.19 (1.90)
Total	6.94 (7.39)	24.75 (11.57)

Note: Data were described by mean (SD).

Abbreviations: 6-BG, baicalein-6-O-glucuronide; 6-BS, baicalein-6-O-sulfate; 7-BG, baicalein-7-O-glucuronide; 7-BS, baicalein-7-O-sulfate; BGG, baicalein-O-diglucuronide; BGGlu, baicalein-O-glucose-O-glucuronide; MeBG, methyl-O-baicalein-6-O-glucuronide; SD, standard deviation.

Therefore, in our PK analysis, we mainly focused on the baicalein, 7-BG, 7-BS, and BGG.

The HL_Lambda_z of baicalein obtained on day 1 and day 10 were 14.91 (9.91) h and 11.29 (4.20) h. The trough concentration of baicalein on days 8–10 showed that a steady-state concentration of baicalein in plasma was achieved after 6 days of repeated dosing. The mean $R_{AUC0-\tau}$ was 2.90 (1.64) and the mean R_{Cmax} was 2.91 (2.58). It indicated that accumulation might occur after multiple administration. Reducing the drug administration frequency may be considered for managing the drug accumulation.

According to the literature, 10 healthy male subjects took 5.2 g baicalin commercial powder orally, and the results of the study on the metabolism kinetics of baicalin and various aglycones in their urine showed that the total cumulative excretion rate of baicalein glucuronide and sulfate in urine at 48 h accounted for 2.9% and 4.3% of the administered dose, respectively. The cumulative excretion rate of wogonin (An O-methylated flavone, a flavonoid-like chemical compound, which is also found in Scutellaria baicalensis.) glucuronide and sulfate in urine for 48 h accounted for 5.9% and 5.7% of the dosage, respectively. The total urine excretion rate of all metabolites of baicalein and wogonin is 7.2% and 11.6%, respectively.[1] The results of this document are similar to the results of the cumulative total excretion rate of ~ 7% after 72 h of oral administration of baicalein 600 mg on the first day of this trial. The 600 mg dose group for multiple administrations showed that the peak urinary excretion rate was five times that of a single administration, and the cumulative excretion rate of the urine segments showed a double peak phenomenon, which was consistent with the previous single-dose trial results. It was speculated that the low recovery rate in urine might be due to the partial excretion of baicalein and its metabolites from the bile into the intestine and enter enterohepatic circulation. Alternatively, a large number of intestinal floras may metabolize baicalein into other undetected metabolites. The higher individual differences may be related to the complexity of the metabolic pathway of flavonoids in vivo and the diversity of related metabolic enzymes.[13]

It had been reported in the literature that the content of baicalein in urine was minimal, but the content of baicalein in the stool was very high.[14] One limitation of this study was that we only analyzed the excretion rate in the urine. After repeated administrations, the clearance rate and apparent distribution volume of baicalein changed, which may be due to changes in metabolic pathways. A more comprehensive study and analysis of the metabolic process in human body will be needed in the future.

Multiple oral administration of baicalein within the dosage range of 200–600 mg was safe and tolerable to healthy Chinese subjects. It had no damage to liver function and

kidney function and may influence triglyceride metabolism. The occurrence and frequency of adverse reactions were not related to the dose. Compared with a single-dose administration, the HL_Lambda_z and T_{max} of baicalein and its metabolites were basically unchanged after multiple administrations. The 7-BS and BGG had higher exposures in vivo, and the exposures of other metabolites were comparable to the original drug baicalein. The accumulation of baicalein and its metabolites was found after multiple administration but not to a potentially toxic level. In conclusion, baicalein tablet was generally safe and well-tolerated in healthy Chinese subjects over the studied dose range.

ACKNOWLEDGEMENTS

The authors are grateful for all those involved in the implementation of the research center. We also thank Chang Liu, Lei Wang, Tingyuan Yang, and Zijian Hu for assistance with text editing that greatly improved the manuscript.

CONFLICT OF INTEREST

L.L. and H.G. wrote the manuscript. R.D. and Z.L. designed the research. R.D., Z.L., L.L., H.G., K.L., and H.L. performed the research. S.H. and J.Y. analyzed the data.

ETHICAL APPROVAL

The protocol, informed consent and other relevant test documents of this trial were approved by the Ethics Committee of Drug Clinical Trials of the Affiliated Hospital of Academy of Military Medical Sciences on November 16, 2011.

INFORMED CONSENT

All subjects obtained informed consent before being included in the study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Li L, Gao H, Lou K, et al. Safety, tolerability, and pharmacokinetics of oral baicalein tablets in healthy Chinese subjects: A single-center, randomized, double-blind, placebo-controlled multiple-ascending-dose study. *Clin Transl Sci*. 2021;14:2017–2024. https://doi.org/10.1111/cts.13063