

Influence of Circadian Variation on the Pharmacokinetics of the Components of da-cheng-qi Decoction

Juan Li, Han-Lin Gong, Yan-Yi Ren, Mei-Hua Wan,
Xi Huang and Wen-Fu Tang*

Department of Integrated Traditional Chinese and Western Medicine,
West China Hospital, Sichuan University, Chengdu, Sichuan Province - 610 041, PR China.

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In the present study, we investigated the effect of dosing time on the pharmacokinetics of components of the Chinese herbal formulation termed da-cheng-qi decoction (DCQD). DCQD was administered orally to rats at a dosage of 20 g/kg under a 12 h:12 h light-dark cycle at 20:00, 00:00, 08:00 and 12:00 hours (0, 4, 12 and 16 hours after light onset). Plasma samples were analyzed for aloe emodin and emodin contents by high performance liquid chromatography (HPLC). The kinetic profiles were best described by a two-compartment model. Linear regression, two-way analysis of variance (ANOVA), non-linear regression analysis, and multiple comparisons of pharmacokinetic data revealed significant dosing time variations. Times to peak concentration (C_{max}) varied from 0.35 to 0.54 hours for aloe emodin and from 0.45 to 0.51 hours for emodin after administration at the selected times. Substantial time-dependent differences in the C_{max} from 4.17 ± 0.82 to 0.23 ± 0.05 mg/L for aloe emodin and from 0.97 ± 0.24 to 0.27 ± 0.05 mg/L for emodin were observed. Furthermore, the area under the time-concentration curve decreased in a time-dependent manner from 7.79 ± 1.91 to 0.52 ± 0.05 mg l⁻¹ h⁻¹ for aloe emodin and from 2.81 ± 0.62 to 0.86 ± 0.18 mg l⁻¹ h⁻¹ for emodin. Findings strongly support a circadian influence on the pharmacokinetics of aloe emodin and emodin after oral dosing with DCQT, with exposure to the herbal components being greater during the rest cycle.

Key words: Da-cheng-qi Decoction, Chronopharmacokinetics, Chinese herbal formula, HPLC.

Da-cheng-qi decoction (DCQD), a well-known traditional Chinese medicine prescription used widely in China and East Asia, is composed of dahuang, houpu, zhishi and mangxiao. The decoction has been used historically as a purgative in the treatment of constipation and for “clearing internal heat” in the stomach and intestine¹. During the past 30 years, DCQD has also been employed to treat acute pancreatitis¹. According to the theory of traditional Chinese medicine, acute pancreatitis is part of the “Yang-Ming-Fu-Shi syndrome” which is best treated between 03:00 and 09:00 hours. However, DCQD is mainly administered to patients with acute pancreatitis during the day as opposed to during the evening or at night. It is possible that

* To whom all correspondence should be addressed.
Tel.: (+86) 28-8542-2556; Fax: (+86) 28-8542-3028
E-mail: wftang900@gmail.com

the pharmacokinetic properties of the components of DCQD may differ as a function of time of administration of the decoction but the clinical consequences of time-dependent variations in its pharmacological foundation have not been evaluated.

In previous studies with rats, the pharmacokinetics of rhein, aloe emodin, and chrysophanol present in DCQD were found to display inter-animal variations². Although diurnal influences may have contributed to such variability⁽³⁾, circadian effects on the pharmacokinetics of components of the herbal formula have not been studied. In this regard, it is of interest that chronopharmacological changes in *Paeoniae Radix* and *Glycyrrhizae Radix* were found to be mainly responsible for variations in the clinical and toxic effects of the herbal formula KST⁴. Unfortunately this study did not examine the influence of the time of administration on the pharmacokinetics of the herbal components.

High performance liquid chromatography (HPLC) methodology to examine the pharmacokinetics of aloe emodin and chrysophanol after oral administration of DCQD to rats was recently developed by our group². The initial goal of the present study was to establish a sensitive and precise HPLC method for the simultaneous measurement of aloe emodin and emodin from DCQD. This method was then used to investigate the chronopharmacokinetics of these components in healthy rats. Circadian-dependent changes in concentration-time profiles and in the pharmacokinetic parameters of aloe emodin and emodin from DCQD were of particular interest.

MATERIAL AND METHODS

Materials and Chemicals

Dahuang (product batch number: 0606013), Houpu (product batch number: 0607029), Zhishi (product batch number: 0610043), and Mangxiao (product batch number: 0708009) were purchased from Chengdu Green Herbal Pharmaceutical Co. Ltd. (Cheng, China). DCQD consists of 6.0 g of the root of Dahuang, 6.0 g of the bark of Houpu, 6.0 g of Zhishi (Immature Bitter Orange), and 6.0 g of Mangxiao. The crude drugs were extracted twice by reflux with boiling distilled water for 1 h; the solution obtained

was then concentrated and spray-dried. To calculate the administered doses of aloe emodin and emodin, their contents in the dried powder of DCQD were quantitated as described previously (Tang *et al.* 2008). The contents of aloe emodin and emodin in the spray-dried extract of DCQD were determined to be 1.71, and 2.5 mg/g, respectively. The dried powder was stored at 4°C until use.

The reference standards for aloe emodin and emodin and the internal standard (IS; 1,8-dihydroxyanthraquinone) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All chemicals were of analytical grade. Methanol was HPLC-grade (Tedia Company Inc., USA). Acetic acid was obtained from Chongqing Chemistry Co. Ltd. (Chongqing, China). Water was triple-distilled and filtered through 0.45 µm Millipore membranes.

EXPERIMENTAL

Twenty-four male Sprague–Dawley rats (body weight, 346 ± 14 g) were selected for this study. Animals were born, housed, fed and handled according to the Animal Ethics Committee guidelines of the animal facility of West China Hospital, Sichuan University after the approval of the study protocol was obtained. Rats were maintained according to the conditions described previously⁶. The animals were acclimatized to the facilities for 3 weeks with 12 h light /12 h dark cycles to adjust their circadian rhythm (the dark cycle occurring between 08:00 and 20:00). Rats were fasted with free access to water for 24 h prior to experiments. The animals were randomly divided into four groups that differed according to the temporal dosing times and referring to the state of activity after light onset: 20:00 pm for immediately inactive (0 hours after light onset; 0 HALO), 00:00 am for resting (4 HALO), 08:00 am for immediately active (12 HALO) and 12:00 pm for active (16 HALO). Blood samples (0.4 ml) were collected through the tail vein at time points of 0 (immediately prior to administration), 0.167, 0.25, 0.33, 0.5, 0.75, 1, 2, 4, 6 and 8 h after a single oral dose. Samples were immediately heparinized and clarified by centrifugation at 3,000 rpm for 10 min. Supernatants were then divided into 0.2 ml aliquots and stored in 1 ml polypropylene tubes at

-20°C until the time of analysis. The voucher specimen of the herbal prescription for this study is an aliquot of the same batch; the code number is 20060625 and the specimen has been deposited in the store room of our laboratory.

Chromatographic Conditions

A Waters 2996 liquid chromatograph system consisting of a photodiode array detector, a Waters 2695 separation module with an automatic sampler, and an on-line degasser coupled with an Empower chromatography analytical workstation was used. The analytical column was a C18 reversed-phase column (5 µm, 150 × 4.6 mm) with an RP18 (5 µm) guard column (both from Dikma). The mobile phase was methanol–0.2% acetic acid (79:21, v/v, pH 4.996) and the flow rate for this phase was 1 ml/min. The detection wavelength was 254 nm. To balance the system, a 10 min lag was maintained between each sampling. The chromatographic separation was performed at 26°C.

Preparation of Plasma Samples

Frozen samples were re-equilibrated to room temperature by incubation for 20 min in a 36°C water bath. A 0.2 ml aliquot of plasma and 1.2 µl of IS (500 µg/ml) were placed in a 1.5 ml polypropylene centrifuge tube and thoroughly homogenized by vortexing. The resulting homogenate was mixed with 80 µl of 2 M hydrochloric acid for 30 s and then extracted with 0.8 ml diethyl ether. After 5 min of mixing by vortex, the preparation was centrifuged at 8,000 rpm for 10 min to achieve separation of phases. The supernatant was transferred to a fresh 1.5 ml centrifuge tube and dried at 40°C in a constant temperature water bath in a fume cupboard. The dried residue was reconstituted in 200 µl of methanol, and 20 µl of this solution was injected into the HPLC system. The same sample handling process was used for assessments of recovery and precision.

Calibration Curves

Stock solutions of aloe emodin with IS (500 µg/ml) and emodin with IS (500 µg/ml) were prepared by dissolving each of the substances in methanol. The calibration curves were constructed based on analysis by HPLC of various concentrations of aloe emodin (16.05, 64.2, 160.5, 642, 1605, 3210 and 6420 ng/ml) and of emodin (3.4, 13.6, 34, 136, 340, 680 and 1360 ng/ml)

added to drug-free plasma samples.

Pharmacokinetic Analyses

The concentration–time data were computer fitted using the “Pharmacokinetics 3p97 Program for Drugs and Statistics” as edited by the Mathematics Pharmacological Committee, Chinese Pharmacological Society. The following pharmacokinetic parameters were calculated: peak concentration (C_{max}), time to maximum plasma concentration (T_{max}), half-life ($t_{1/2}$), area under the concentration–time curve (AUC_{0-8h}), clearance (CL), and elimination rate constant. Additional parameters were also investigated. The mean retention time (MRT) was calculated using the statistical moment method of non-compartmental pharmacokinetic analysis.

Statistical Evaluations

Linear regression, two-way analysis of variance (ANOVA), non-linear regression analysis, and multiple comparisons of the parameters were used. A probability level of $\alpha < 0.05$ was considered significant.

RESULTS

Analytical Variables

Chromatography

Fig. 1 presents HPLC chromatograms of extracts of (A) a plasma control sample, (B) a plasma control containing IS and reference standards for aloe emodin and emodin, and (C) a plasma sample collected at 1 h following administration of DCQD. Based on the chromatographic behavior of the pure reference and internal standards, the peaks labeled 1, IS, and 2 in (B) and (C) and with retention times of 4.28, 8.06, and 9.64 min were identified as aloe emodin, 1,8-dihydroxyanthraquinone, and emodin, respectively. Under the chromatographic conditions described, complete resolution was achieved and no interfering peaks were observed within the time frame in which aloe emodin, IS, and emodin were detected. The rapidity of the overall procedure, which required a run-time of 11 min for each analysis, is consistent with efficient sample preparation and clean-up methodology. The use of methanol and acetic acid for the mobile phase resulted in good baseline separation and satisfactory peaks. Based on a signal-to-noise ratio of 3, the limits of detection for aloe emodin and

emodin were 3.2 and 7.7 ng/ml, respectively.

Linearity

The calibration curve for aloe emodin was linear ($r^2 = 0.995$) over the concentration range of 16.05–6420 ng/ml. The linear regression equation of $y = ax + b$ (where x is the peak area ratio and y the concentration of analyte) was obtained with $CL_{95\%}$ (a) = 0.223 ± 0.023 and $CL_{95\%}$ (b) = 0.027 ± 0.057 (ng/ml). For emodin, the calibration curve was linear over the concentration range of 3.4–1360 ng/ml ($r^2 = 0.994$); a regression equation of $y = ax + b$ was obtained with $CL_{95\%}$ (a) = 0.211 ± 0.011 and $CL_{95\%}$ (b) = 0.028 ± 0.006 (ng/ml). These concentration ranges were found to be representative of the concentrations observed during the analysis of collected plasma samples. Considerable linearity was obtained, with precision and accuracy comparable to that reported by Tang et al. ⁽²⁾.

Efficiency of Extraction Procedures

Drug- free plasma samples were spiked with three different concentrations of aloe emodin and emodin, with fixed amounts of IS added to the samples for normalization. Recovery was calculated using the internal standard method. The method for simultaneous determination of aloe emodin and emodin required only 0.2 ml plasma that had been extracted using a simple procedure involving diethyl ether. The mean recoveries of aloe emodin and emodin from rat plasma were 94.25% and 93.83 %, respectively, with mean

relative standard deviations of 7.47 % and 5.08%, respectively.

Reproducibility, Precision, and Accuracy

The reproducibility of the method was evaluated by examining both intra- and inter-day variances. The intra-day variation was assessed by analyzing five standard concentrations of aloe emodin and emodin within a single day. The inter-day variation was evaluated by analyzing three standard curves for these substances obtained on three non-consecutive days. Precision was expressed as intra- and inter-day coefficients of variation (CV). The intra-day CVs for aloe emodin and emodin were $5.0 \pm 0.1\%$ and $5.3 \pm 1.3\%$, respectively, while the inter-day CVs were $7.5 \pm 1.9\%$ and $5.1 \pm 1.8\%$, respectively. Accuracy was defined as the mean CV of all concentrations from the theoretical value ($CV = 5.6 \pm 1.3\%$).

Kinetic Studies

The plasma concentration-time profiles of dosing time kinetics for four different times of DCQD administration were described by a two disposition model (Fig. 2). Over the four dosing times, the mean half-lives for the distributive phases varied from 0.69 to 1.68 h for aloe emodin and from 0.2 to 5.01 h for emodin whereas mean half-lives for the elimination phases varied between 4.9 and 5.91 h for aloe emodin and between 7.57 and 20.05 h for emodin. These findings indicate that aloe emodin and emodin from DCQD are rapidly distributed into extra-vascular tissues and

Table 1. Pharmacokinetic Parameters for Aloe Emodin for Four Different DCQD Dosing Times ($n = 6$)

Aloe Emodin	0 HALO (20:00)	4 HALO (0:00)	12 HALO (8:00)	16 HALO (12:00)	P^a
Tmax (h)	0.35 ± 0.09	0.5 ± 0.05	0.47 ± 0.08	0.54 ± 0.07	0.0015
$t_{1/2\alpha}$ (h)	1.04 ± 0.13	1.68 ± 0.36	0.69 ± 0.17	1.10 ± 0.29	0.0000
$t_{1/2\beta}$ (h)	5.49 ± 0.89	5.91 ± 0.85	4.90 ± 0.65	5.87 ± 0.69	0.1235
CL/F (L/h/kg)	3.76 ± 0.49	7.23 ± 1.15	17.25 ± 3.03	72.72 ± 9.23	0.0000
AUC ₍₀₋₁₂₎ (mg/L*h)	7.79 ± 1.91	2.59 ± 0.54	1.68 ± 0.39	0.52 ± 0.05	0.0000
Cmax (mg/L)	4.17 ± 0.82	0.77 ± 0.19	0.66 ± 0.11	0.23 ± 0.05	0.0000
k_{10} (L/h)	1.25 ± 0.22	1.29 ± 0.21	0.59 ± 0.13	0.71 ± 0.16	0.0000
k_{12} (L/h)	2.36 ± 0.34	1.84 ± 0.13	1.73 ± 0.27	0.77 ± 0.11	0.0000
k_{21} (L/h)	0.44 ± 0.12	0.68 ± 0.11	0.71 ± 0.14	0.43 ± 0.11	0.0000
k_a (L/h)	13.23 ± 2.34	2.53 ± 0.32	10.05 ± 2.05	2.82 ± 0.29	0.0000
MRT _(0-t) (h)	2.88 ± 0.42	4.14 ± 0.57	2.986 ± 0.31	3.82 ± 0.69	0.0008

^aP values are the results of ANOVA among the four dosing times.
HALO, hours after light onset.

then eliminated. As shown in Tables 1 and 2, the apparent first-order elimination rate constant (k_{10}) for aloe emodin in the light period (0 and 4 HALO) was much bigger than that in the dark period (12 and 16 HALO), revealing important variations in relation to dosing time. However, the difference in k_{10} values between the light and dark periods for emodin was not as great as that for aloe emodin. The inter-compartmental rate constants k_{12} (distribution rate constant from central to peripheral compartment) and k_{21} (distribution rate constant from peripheral to central compartment) were both dependent on dosing time. Determination of the k_{12}/k_{21} ratio as a function of the dosing time indicated that exchanges between the central and the peripheral compartments are likely to be dosing time-dependent. As shown in Fig. 3, aloe emodin was most strongly retained in the peripheral compartment when administered in the immediately inactive period (20:00; 0 HALO). These time-related variations are similar to the circadian variations in the pharmacokinetics of nifedipine observed after administration of a single oral dose to rats⁷. Emodin was more strongly retained in the peripheral compartment when administered during the periods of 0 and 4 HALO. This observation may constitute the pharmacokinetic foundation underpinning the clinical benefits of administration of DCQD during the daytime. In addition to these kinetic findings, however, determinations of the concentrations and

pharmacodynamic actions of the components of DCQD in various tissues will be required to optimize the therapeutic, while minimizing the toxic, actions of the decoction.

Circadian Changes in Plasma Concentrations of Aloe Emodin and Emodin from DCQD

The mean plasma concentration-time profiles for aloe emodin and emodin after oral administration of a single dose of DCQD at four different dosing times are presented in Fig. 2. All four curves displayed two-model kinetics for the two components of DCQD in rats. The plasma concentrations for rats dosed at 12 and 16 HALO (dark period) were lower than those dosed at 0 and 4 HALO (light period). The highest concentrations were obtained at 0 HALO (immediately inactive) while the lowest was at 16 HALO (active). Upon a change from the rest cycle in the light period to the active cycle in the dark period, gradual decreases in the plasma concentrations of both substances were observed. Differences in mean plasma concentrations among the four dosing times were statistically significant. Daytime to nighttime differences in plasma concentrations of aloe emodin and emodin following oral administration of DCQD were also observed. However, this finding differs from that for orally administered nifedipine which is reported to be present at higher plasma concentrations at 12 HALO⁷.

Table 1 and Table 2 present the pharmacokinetic parameters for aloe emodin and emodin, respectively, from DCQD at four different

Table 2. Pharmacokinetic Parameters for Emodin for Four Different DCQD Dosing Times ($n = 6$)

Emodin	0 HALO (20:00)	4 HALO (0:00)	12 HALO (8:00)	16 HALO (12:00)	P^a
Tmax (h)	0.45 ± 0.04	0.51 ± 0.07	0.5 ± 0.07	0.47 ± 0.05	0.0011
$t_{1/2\alpha}$ (h)	2.25 ± 0.31	0.59 ± 0.13	0.20 ± 0.04	5.01 ± 0.62	0.000
$t_{1/2\beta}$ (h)	7.62 ± 1.59	20.05 ± 3.245	7.57 ± 1.38	17.38 ± 2.47	0.000
CL/F (L/h/kg)	12.75 ± 1.62	16.64 ± 2.44	19.33 ± 4.74	23.94 ± 3.07	0.0001
AUC ₍₀₋₁₂₎ (mg/L*h)	2.81 ± 0.62	1.65 ± 0.32	1.46 ± 0.31	0.86 ± 0.18	0.0000
Cmax (mg/L)	0.97 ± 0.24	0.63 ± 0.19	0.53 ± 0.18	0.27 ± 0.05	0.0000
k_{10} (L/h)	0.37 ± 0.03	0.20 ± 0.01	0.73 ± 0.09	0.23 ± 0.02	0.0000
k_{12} (L/h)	1.09 ± 0.12	1.07 ± 0.11	1.29 ± 0.14	0.91 ± 0.11	0.0003
k_{21} (L/h)	0.33 ± 0.07	0.43 ± 0.10	0.79 ± 0.14	0.48 ± 0.05	0.0000
MRT _(0-t) (h)	2.76 ± 0.25	4.94 ± 0.35	3.12 ± 0.32	5.08 ± 0.46	0.0000

^aP values are the results of ANOVA among the four dosing times.
HALO, hours after light onset.

dosing times. $C_{\max}$ and AUC values were the lowest for rats dosed during the resting period of 16 HALO, although the highest $C_{\max}$ and AUC values were not observed for rats dosed during the active period of 4 HALO. The $C_{\max}$ and AUC values for aloe emodin for rats dosed in the light cycle were higher than those for rats dosed in the dark cycle. Performance of the ANOVA test revealed statistically significant differences in the pharmacokinetics of aloe emodin and emodin among the four different dosing times, suggesting a generalized circadian time-dependency of $C_{\max}$ and AUC. By contrast, the $T_{\max}$ for aloe emodin was longest for rats dosed at 16 HALO and shortest for rats dosed at 0 HALO whereas $T_{\max}$ values for emodin were significantly different among the four dosing times. These findings indicate a widely variable circadian time dependency for these substances. Accordingly, the total clearance (CL) values varied as a function of the temporal dosing

time from 3.76 ± 0.49 to $72.72 \pm 9.23 \text{ l h}^{-1} \text{ kg}^{-1}$ for aloe emodin and from 12.75 ± 1.62 to $23.94 \pm 3.07 \text{ l h}^{-1} \text{ kg}^{-1}$ for emodin. These changes agree with the observed changes in $C_{\max}$ and AUC values at the four different dosing times. CL values for the light period were lower than those for the dark period. MRT values varied between $2.88 \pm 0.42 \text{ h}$ and $4.14 \pm 0.57 \text{ h}$ for aloe emodin and between $2.76 \pm 0.25 \text{ h}$ and $5.08 \pm 0.46 \text{ h}$ for emodin. The CL and MRT values for aloe emodin and emodin both clearly displayed a circadian time dependency in this study.

DISCUSSION

The present study is the first to identify dark/light cycle differences in the pharmacokinetics of aloe emodin and emodin following administration of DCQD to rats. The observed circadian variations in kinetics could be

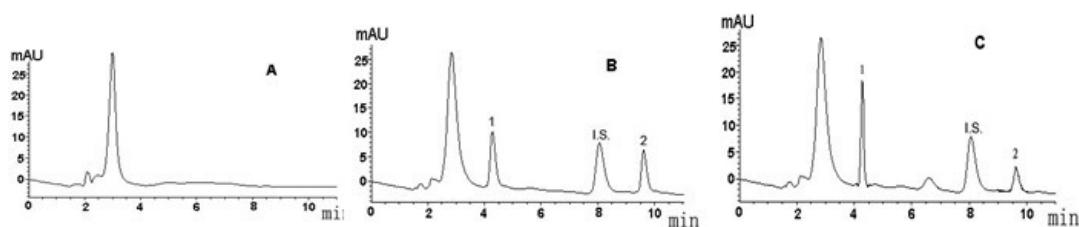


Fig. 1. Typical HPLC chromatograms for the determination of aloe emodin and emodin in plasma samples. (A) drug-free control plasma sample; (B) plasma sample containing internal standard (IS) and reference standards for aloe emodin (1) and emodin (2); (C) plasma sample collected at 1 h following oral administration of DCQD

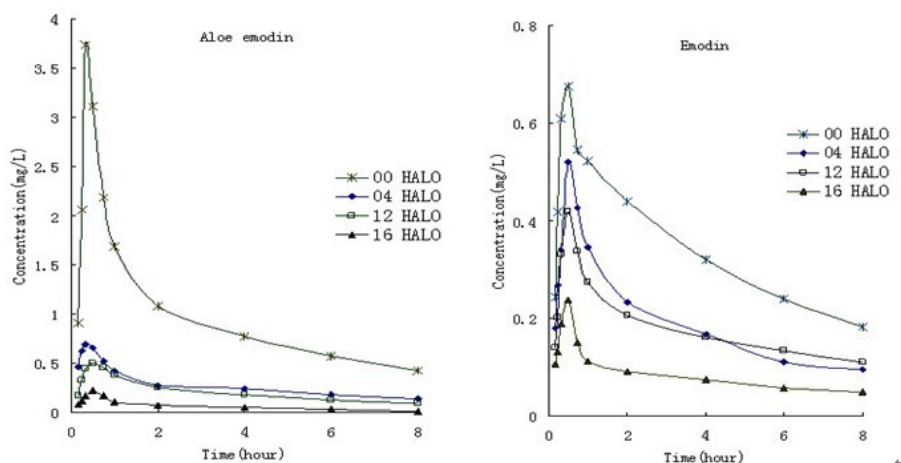


Fig. 2. Plasma concentration-time curves for aloe emodin and emodin after oral administration of DCQD to rats at four different dosing times. DCQD was administered at 20g /kg body weight. Each point with its corresponding error bar represents the mean $\pm$ SD ($n = 6$)

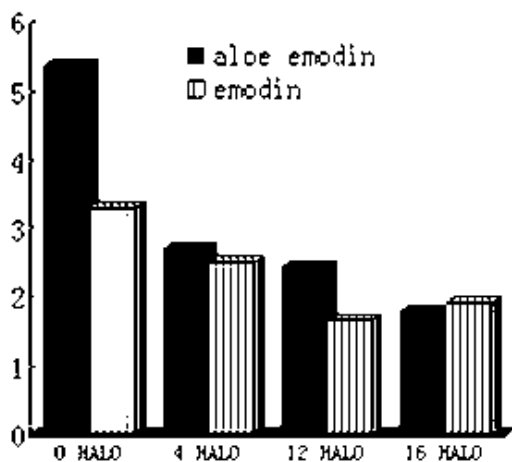


Fig. 3. Variations of the inter-compartmental rate constant ratio k_{12}/k_{21} as a function of dosing time expressed as hours after light onset (HALO). White and black zones (horizontal axis) correspond to light (rest) and dark (active) spans, respectively

due to corresponding time-dependent changes in conditions and/or variables involved in the absorption, distribution, metabolism and excretion of the components of this Chinese herbal formula. Factors known to influence absorption include stomach pH, gastric emptying, gastrointestinal motility and reactivity, blood flow to the liver and intestines, gut flora, herbal component interactions, and “first-pass” effects in the stomach, intestine, and liver^{8,9}. Unfortunately, the circadian influences of most of these factors on the absorption of herbal components in rats remain unclear. Circadian changes in acid secretion and gastric mucosal blood flow may contribute to the time-dependent absorption of certain herbal components¹⁰. Circadian changes in gastric pH would significantly affect the absorption of tart or basylous chemicals, such that circadian changes in pharmacokinetics would be observed. It should also be noted that blood flow to the intestine, liver and kidney in rats has been found to be maximal during the active period and minimal when the animals are asleep¹¹, corresponding to the maximal ratio for exchange¹². However, this concept is not in agreement with the observed exchanges of aloe emodin and emodin between the central and peripheral compartments; the k_{12}/k_{21} ratios were higher for the rest times of 0 and 4 HALO as

compared to ratios for the active times of 12 and 16 HALO. Higher k_{12}/k_{21} ratios were observed in the light cycle, with higher C_{max} and AUC values. Finally, circadian changes in gastrointestinal melatonin have been proposed to promote dark/light cycle differences in the pharmacokinetics of components of Chinese herbal formulations¹³. However, no studies to support this hypothesis have been published.

The variables involved in the distribution, metabolism, and excretion of the components of Chinese herbal formulations remain to be established. Although many factors possess the potential to promote circadian changes in drug distribution and excretion, no such factors have been identified^{14,15}. Regarding the importance of metabolism, circadian effects of monomers derived as a result of metabolism of components of certain Chinese herbal preparations have been linked with pathophysiological changes in the nervous system¹⁶⁻¹⁸, however, the relevant pharmacokinetics was not explored in these studies. It is recommended that further consideration be given to time-dependent alterations in the pharmacokinetic properties of components of traditional Chinese herbal formulations. A better understanding of the influence of circadian rhythm on the pharmacokinetics of components of herbal preparations should serve to guide the clinical chronotherapy of such remedies. In summary, significant circadian differences in the pharmacokinetics of aloe emodin and emodin were observed when DQCD was administered orally to rats at different times within a single day. The distribution phases of aloe emodin and emodin were both altered. Their $t_{1/2\alpha}$ values were both dosing time-dependent, and their elimination phase $t_{1/2\beta}$ values were also found to change in a time-related fashion. The half-time of the elimination phase for aloe emodin was time-dependent whereas that for emodin was not. These findings reveal that time-dependent effects on the pharmacokinetics of components from the same herbal formula may differ for each component, even though the AUC, C_{max} , and CL values for these components may be altered similarly as a function of circadian variation. Dosing time-dependent variations in inter-compartmental rate constants could guide, at least in part, clinical usage of these formulations during the day.

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