Rational Daily Administration Times of Yinchenhao Decoction in Rats with Jaundice Based on PD/PK

LV Jun-lan[1, 2], JIN Shi-ying[1, 2], YUAN Hai-long[1*], HAN Jin[1], FU Shan-shan[1, 2], JIN Shi-xiao[1, 2], GUO Jing-jing[1, 2], XIAO Xiao-he[1*]

1. China Military Institute of Chinese Materia Medica, 302 Military Hospital, Beijing 100039, China
2. School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 611731, China

Abstract: **Objective** To study the rational daily administration times of Yinchenhao Decoction (YCHD) when it was used to treat experimental jaundice in rats based on pharmacodynamics/pharmacokinetics model. **Methods** Rats were modeled by 4% 1-naphthylisothiocyanate (75 mg/kg) for 48 h, then YCHD was drenched with doses of 0.324 g/kg (extract, calculated with the clinical dosage) once, 0.162 g/kg twice, and 0.108 g/kg thrice a day, respectively. The total bile and the flow rate of bile were observed after the first administration; Blood samples collected from the orbital sinus at different intervals were used to investigate the levels of liver enzymes (ALT and AST) and bilirubins (TBIL and DBIL), and determine the concentration of 6,7-dimethoxycoumarin (DME) in the plasma using UPLC at the same time, then we obtained the time-effect and time-dose curves. The rational daily administration times of YCHD when treating experimental jaundice were determined based on the comprehensive analysis of time-effect and time-concentration relationships. **Results** Within 10 h the total bile of rats which were administered once daily (G1) was 1.65 and 1.33 times higher than that of twice and thrice (G2 and G3) a day, respectively, and the four biochemical indexes (TBIL, ALT, DBIL, and AST) of G1 decreased faster than those of G2 and G3 ($P < 0.05$). On the other hand, the blood drug level of DME when administrated once daily could maintain at a higher level for a longer time, and its C_{max} and $AUC_{0\rightarrow t}$ were higher than those of G2 and G3, which might be the main reason why its effect was the most significant. **Conclusion** It is more appropriate to administrate once daily when YCHD is used to treat jaundice.

Key words: bilirubins; 6,7-dimethoxycoumarin; jaundice; pharmacodynamics/pharmacokinetics; Yinchenhao Decoction
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Introduction

The administration method of Chinese materia medica (CMM), including administration dosage, times, and intervals, as a key scientific issue of CMM, is directly related to the clinical efficacy and safety. In resent years, attention has been paid to the exploration of "dosage and dose-effect relationship", but little concern was taken on the rational daily administration times. There was lack of modern scientific evidence about whether the prescriptive daily administration times of oral medicine was reasonable or not, because it often worked empirically, with certain arbitrariness and subjective nature, for example, when the prescriptive dosage of a medicine was four pieces a day, the possible administration times was twice daily; and if a medicine was prescripted to be nine pieces a day, it was possible suggested to be taken for three times a day. At present, study on the rational daily administration times is almost blank (Yuan and Xiao, 2011).

Yinchenhao Decoction (YCHD) is one of the most frequently used prescriptions in the long history of traditional Chinese medicine (TCM) practice. The prescription consists of three medicinal herbs, *Artemisiae Scopariae Herba* (Yinchenhao), *Gardeniae*

* Corresponding author: Yuan HL Address: China Military Institute of Chinese Materia Medica, 302 Military Hospital, Beijing 100039, China
Tel: +86-10-6693 3367 E-mail: yhlpharm@126.com
Xiao XH Address: China Military Institute of Chinese Materia Medica, 302 Military Hospital, Beijing 100039, China Tel: +86-10-6693 3322
E-mail: pharmacy302@126.com
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Fructus (Zhizi), and *Rhei Radix* et *Rhizoma* (Dahuang), and has been widely used to treat acute hepatitis with jaundice (Cheng *et al*, 2008). It has the effect of clearing heat, withdrawing jaundice, detoxication, eliminating dampness, and normalizing gallbladder. So it has long been used in China as an anti-inflammatory, antipyretic, and choleretic agent for liver disorders and jaundice in clinic (Lee *et al*, 2009). YCHD has been recognized by some practitioners and researchers for its beneficial effects in treating liver diseases (Arai *et al*, 2004). The reasons why we chose YCHD for researching were as follows: (1) Its clinical effect is pronunced and significant; (2) The pharmacodynamic (PD) index could be easily determined; and (3) Its effective substances were relatively clear. In clinical applications, the general administration method of YCHD was considered as 54 g (crude drug) per person a day, subpackaged into small bags after decoction, taken one bag each time for three times everyday, and this regimens seemed to be accepted by both doctors and patients, however, there was no scientific basis for whether it was effective and reasonable or not.

The administration times of Western medicines could be determined by the pharmacokinetics (PK) characteristics because they are generally composed with single-component and their PK characteristics are definite to some extent (Yu *et al*, 2009; Li *et al*, 2011). But the efficacy of CMM usually came from the synthetic actions of multi-components, even a single medicine was a small compound recipe, the PK data of some single components might be not enough to represent the behavior of CMM in the body (Liu, 2003). So the classical PK model might not be suitable to study the administration times and dosage requirements of CMM. So we innovatively adopted the PD/PK model in this study according to the perplexing characteristics of CMM, and it proved to be more scientific and practical. In this model, the pharmacologic effects of drugs were regarded as the research core, and the PK of some effective ingredients were as assistance. It highlighted the role of PD compared with the PD/PK model. When the PK coincided with the pharmacologic effects, we could directly define the administration methods of the drug through the PK or PD parameters, but if they did not coincide with each other, the administration times of drugs might be determined mainly on the basis of the PD parameters.

Materials and methods

Apparatus

Ultra-high performance liquid chromatography (UPLC) (Waters Acquity, USA); Waters Acquity BEH C_{18} Chromatographic column (50 mm × 2.1 mm, 1.7 μm); Vortex mixer was bought from Haimen Kylin-Bell Lab Instruments Co., Ltd. (Haimen, China); Mindray automatic biochemical analyzer was purchased from Shenzhen Mindray Medical International Ltd. (Shenzhen, China); Centrifuge was purchased from Shanghai Anting Scientific Instrument Factory (Shanghai, China); Electronic balance was bought from Sartorius Stedim Biotech (Beijing) Co., Ltd. (Beijing, China).

Materials

Artemisiae Scopariae Herba, *Gardeniae Fructus*, and *Rhei Radix* et *Rhizoma* were bought from Lvye Pharmaceutical Co., Ltd. (Beijing, China); 6,7-Dimethoxy-coumarin (DME) standard was purchased from National Institute for Food and Drug Control (Beijing, China); 1-Naphthylisothiocyanate (ANIT) of 95% purity was bought from Sigma Company; Test kits for liver enzyme were purchased from Shenzhen Mindray Medical International Ltd. (Shenzhen, China); Heparin sodium was bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China); Urethane was bought from Sinopharm Chemical Reagent Co., Ltd., Lot. (Shanghai, China); Olive oil of chemical pure was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China); Methanol and methyl cyanides of HPLC grade were purchased from Promptar Co., Ltd. (California, USA); pure water.

Preparation of YCHD

Yinchenhao (200 g), Zhizi (100 g), and Dahuang (60 g) were put into a round-bottomed flask, then added with 10 times distilled water, heated to boiling continuously for 1 h. The decoction was then percolated to obtain filtrate, and the residues were reboiled for twice more. The collected filtrate was then poured together and concentrated under reduced pressure. The concentrated liquid was further dried in vacuum to get the YCHD extract. The yield of the YCHD extract was 28.6% and the concentration of DME was 16 mg/g. It was stored in dessicator until use.

Animals and grouping

Wistar rats of SPF grade weighing from 200 to 220 g were supplied by Military Medical Science Academy of the PLA [SCXK-(army)2007-004], housed in standard laboratory conditions, and had free access to water and commercial rat chow. They were maintained under a constant 12 h light-dark cycle at an environment temperature of 21－23 ℃ and relative humidity of (55 ± 5)%. They were randomly divided into five groups, blank group (B) and model group (M): ig administrated with 0.9% sodium chloride after modeling, group of one administration a day (G1), ig administrated with YCHD (0.324 g/kg at 9:00); group of two administrations a day (G2): (0.162 g/kg at 9:00 and 13:00); and group of three administrations a day (G3): (0.108 g/kg at 9:00, 13:00, and 17:00).

PD of cholaneresis

In addition to the blank group, rats in M, G1, G2, and G3 groups were given 4% ANIT olive oil solution (75 mg/kg) to establish high jaundice model (Wang *et al*, 2007), and 48 h after modeling, 0.9% sodium chloride or YCHD were ig administrated to them followed by the doses described above. After the first dose, all rats were ip injected with 10% urethane to be anesthetized (1o mL/kg), then laparotomy was proceeded after their anesthesia, the choledochus was separated carefully, pluged in scalp needle, fixed with medical suture, and led out of the abdominal wall, and the hourly bile flow and total bile were recorded (Yu *et al*, 2003). At the 4th hour, the second doses of G2 and G3 were given, and at the 8th hour, the third dose of G3 was given.

PD of reducing jaundice

The model and administration methods were the same as those described above. Blood samples were collected from the orbital sinus pre-dose (0 h) and 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h post-dose into tubes, simultaneously two tubes at each time point. One was centrifuged immediately at 8000 r/min for 10 min at 4 ℃ to yield serum, and an aliquot of 100 μL serum sample was transferred into a new tube and diluted with 400 μL of 0.9% sodium chloride. Then the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), and direct bilirubin (DBIL) activities were measured according to the instructions of the assay kits by automatic biochemical analyzer; The other tube of blood which was anticoagulated by heparin sodium was centrifuged at 8000 r/min for 10 min at 4 ℃ to yield plasma, stored in fridge at −80 ℃ for use.

PK research of DME

Plasma sample process After the frozen plasma samples were thawed at room ternperature, 200 μL plasma sample was transferred into a tube and then 1 mL methanol was added to precipitate plasma protein as well as extract the DME from plasma by vortex-mixing for 30 s and centrifuging at 8000 r/min for 10 min at 4 ℃. The organic supernatant was transferred into another tube and evaporated to dryness under a gentle N_2 stream at 40 ℃ in water bath. The residue was reconstituted in 200 μL of methanol. After centrifuging at 8000 r/min at 4 ℃ for 10 min, 10 μL of supernatant was used for UPLC analysis (Fang, Li, and Watanabe, 2003).

Preparation of standard solutions The primary stock solutions of DME standard (2.102 mg/mL) were prepared by dissolving appropriate amount (2.102 mg) of reference substance in methanol, then diluted serially in methanol to yield serial standard working solutions.

Conditions of UPLC Waters Acquity BEH C_{18} column (50 mm × 2.1 mm, 1.7 μm), mobile phase methanol-water (45:55), column temperature 30 ℃, flow rate 0.3 mL/min, detection wavelength 343 nm, and injection volume 10 μL.

Method validation

(1) Specificity DME standard solution, blank plasma, blank plasma spiked with DME, and the plasma samples after drug administration were determined by UPLC according to the chromatographic conditions described above.

(2) Linear correlation DME reference substance methanol (2.102 μg/mL) 2, 10, 50, 200, 400, and 800 μL were precisely imbibed into a centrifuge tube with plug, and 200 μL blank plasma was added after the nitrogen dryness, then vortex mixed for 30 s to prepare standard plasma samples with DME concentration of 0.021 02, 0.1051, 0.5255, 2.102, 4.204, and 8.408 μg/mL, respectively. They were determined by UPLC and the plasma concentration (x) was taken with the peak area (y) for linear regression to get the standard curve equation.

(3) Precision Plasma samples with DME

standard concentration of 2.102, 4.204, and 8.408 μg/mL were preparaed according to the "Plasma sample process"; The samples of each concentration were determined for five times to calculate the intra-day precision and continuously determined for 3 d to calculate inter-day precision.

(4) Recovery DME standard solution was added into 100 μL blank plasma to get the plasma concentration of 2.102, 8.408, and 33.632 μg/mL, According to "Plasma sample process", sample (10 μL) was injected into UPLC for analysis, the peak area of DME (A) was recorded; DME of the same concentration was prepared with methanol, the peak area of DME (A_0) was determined and recorded, A and A_0 were compared to calculate the extraction recovery of DME.

Data analyses Statistical analyses were performed with the SPSS 17.0 software. Data were subjected to a one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) post-hoc test, and differences were considered statistically significant at $P < 0.05$. PK analysis was performed with DAS 2.1.1 software.

Results

Total amount of bile

The amount of bile was measured each hour after ig administration of YCHD in five different groups (Fig. 1). There was significant difference ($P < 0.01$) between groups M and K, and it proved to be a successful model in this experiment; The total bile of group G1 was higher than that of group M with significant difference, which indicated that the treatment with YCHD solution (0.324 g/kg) once daily could increase the total bile of rats within 10 h. The results showed significant difference among groups G2, G3, and M. The total bile within 10 h of rats which were administrated once daily was 1.65 and 1.33 times higher than those of two and three administrations a day, respectively.

Hourly bile flow rate

The hourly bile flow in five different groups was shown in Fig. 2. Compared with group M, administration with YCHD for different times could lead to increase of bile flow differently, G1 showed a dramatic increase in bile flow in the first 3 h, then the bile flow began to decrease sharply; Bile flow in G2 group increased significantly in the first 4 h, but got the lowest at the 5th hour, then continued to be lower than that in group M; Bile flow in G3 group has a certain increase, but showed no significant difference compared with that in group M.

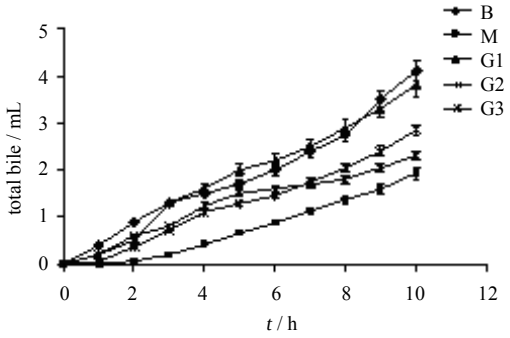

Fig. 1 Total bile-time curves ($\bar{X} \pm s$, $n = 8$)

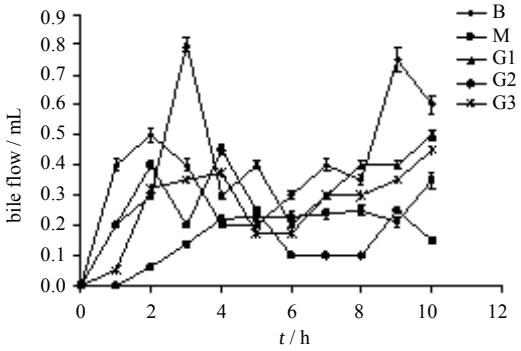

Fig. 2 Hourly bile flow-time curves ($\bar{X} \pm s$, $n = 8$)

TBIL level in serum

The TBIL levels in five different groups were shown in Fig. 3. Compared with group B, the TBIL level of group M was significantly increased, and kept at a high level within 24 h after being modeled with ANIT; There was a certain reduce of G1, G2, and G3, and a significant difference ($P < 0.05$) compared with group M. The TBIL level of G1 decreased rapidly by 97.48% and then reached the normal level within 24 h, and there was significant difference compared with G2 and G3 ($P < 0.05$). The TBIL levels of G2 and G3 were reduced by 86.43% and 86.73% within 24 h, and there was no significant difference between the two groups ($P > 0.05$).

ALT level in serum

The ALT levels in five different groups were shown in Fig. 4. It indicated that the ALT level of group M was kept steady at a high level within 24 h ($P < 0.01$) compared with that of group B; The ALT level of G1

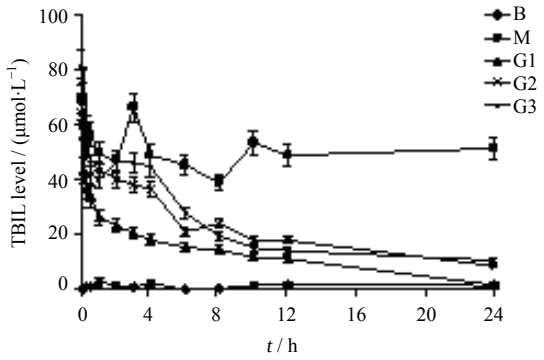

Fig. 3 TBIL level-time curves ($\bar{X}$ ± *s*, *n* = 8)

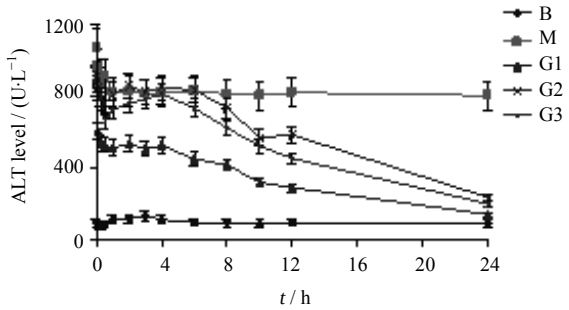

Fig. 4 ALT level-time curves (*n* = 8)

decreased rapidly by 83.80% with less fluctuation within 24 h; ALT levels of G2 and G3 showed a certain reduce compared with that of group M (decreased by 73.81% and 78.10%, respectively), but there was obvious fluctuation.

AST level in serum

The AST levels in five different groups were shown in Fig. 5. It showed that all the AST levels of G1, G2, and G3 had rapid decreases at the beginning and then upturned. AST level of group G1 decreased rapidly by 79.98% within 24 h with less fluctuation, it showed significant difference with G3 ($P < 0.05$), while had no significant difference with G2.

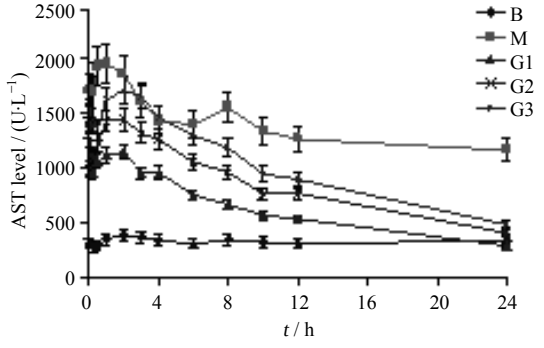

Fig. 5 AST level-time curves ($\bar{X}$ ± *s*, *n* = 8)

DBIL level in serum

The DBIL levels in five different groups were shown in Fig. 6. It was indicated that the DBIL levels of groups G1 and G2 were significantly reduced compared with that of group M, while there was no significant difference between groups G3 and M ($P > 0.05$). The DBIL level of group G1 was significantly lower than those of groups G2 and G3 ($P < 0.05$) within 24 h.

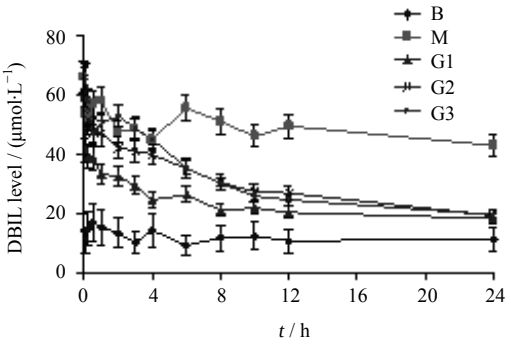

Fig. 6 DBIL level-time curves ($\bar{X}$ ± *s*, *n* = 8)

Method validation

Fig. 7 shows the typical chromatograms of blank plasma, DME standard, plasma sample spiked with DME, and a plasma sample obtained at 15 min after the first administration. It was indicated that under the chromatographic conditions of this experiment, there was no endogenous interference at the retention position of DME; The method was validated and found to be linear over the concentration range of 0.021 02— 8.408 μg/mL ($r^2 = 0.998$) using ($1/C^2$) weighted least squares regression; The precisions of the intra- and inter-day RSD values of DME were 3.28% and 4.57%, respectively ($n = 8$); The mean extraction recoveries of the three concentration levels of DME in plasma were 82.42%, 79.38%, and 74.65%, respectively, and all of them were eligible.

PK in rats

The mean plasma concentration-time curves and the PK parameters were shown in Fig. 8 and Table 1, respectively.

DME could be detected in blood only 5 min after administration of YCHD solution, and the plasma concentration reached the C_{max} at 15 min, which indicated that DME could be rapidly absorbed into blood and developed pharmacological effects. The relationship of C_{max} among different groups was G1 >

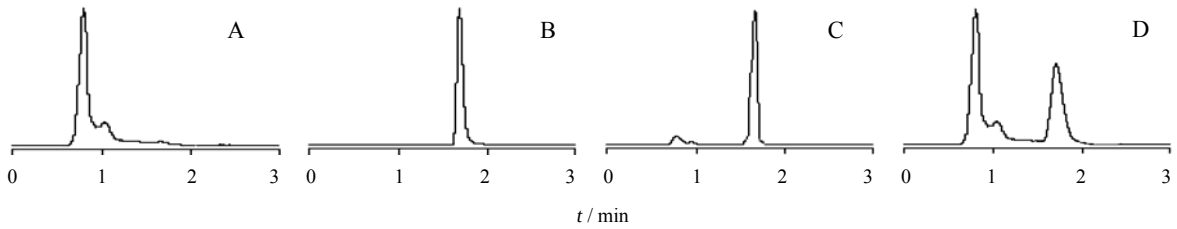

Fig. 7 Chromatograms of blank plasma (A), DME refenence substance (B), blank plasma spiked with DME reference subatance (C), and plasma sample (D)

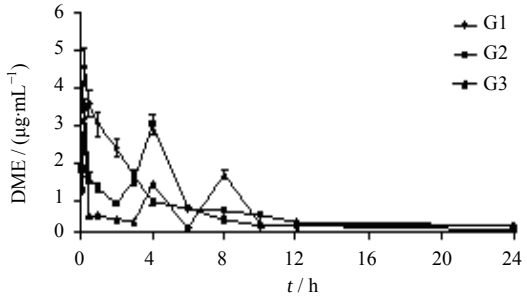

Fig. 8 Mean plasma concentration-time curves of DME after administration of YCHD ($\bar{X} \pm s$, n = 8)

Table 1 PK parameters of DME in rats after administration of YCHD (n = 8)

Parameters	Unit	G1	G2	G3
$t_{1/2\alpha}$	h	10.879	18.650	6860.791
V1/F	$L \cdot kg^{-1}$	7.311	1.481	0.066
CL/F	$L \cdot h \cdot kg^{-1}$	0.279	0.415	0.450
AUC_{0-t}	$mg \cdot L \cdot h^{-1}$	16.469	12.068	10.751
$AUC_{0-\infty}$	$mg \cdot L \cdot h^{-1}$	18.584	12.489	11.524
K_{10}	h^{-1}	0.038	0.280	6.853
K_{12}	h^{-1}	0.022	0.278	6.853
K_{21}	h^{-1}	0.003	0.035	0
k_a	h^{-1}	0.533	0.043	30.601
$t_{1/2}k_a$	h	1.300	16.271	0.023
t_{lag}	h	3.441	0	0.133

G2 > G3, the concentration of DME decreased rapidly after the peak; The curves of groups G2 and G3 have two and three peaks, respectively, but their plasma concen- tration fluctuated in a lower range because of a smaller amount of single dose.

The above results showed that the C_{max}, $AUC_{0\rightarrow t}$, and $AUC_{0\rightarrow 24\ h}$ of G1 were all higher than those in groups G2 and G3, indicated a preferable relative bioavailability.

It could be concluded that it is better to take YCHD once daily in the treatment of experimental jaundice according to synthetical analysis on time-effect and time-concentration (PD/PK) relationships. When administrated once daily, the increase of bile flow and reduction of the biochemical indexs in serum had a significant advantage and also demonstrated that the concentration of DME (the transitional component in blood) maintains at a higher level for a long time, and the relative bioavailability was higher than that of the group administrated twice or thice a day.

Discussion

The "administration times of CMM" have always been an important problem in the standardization research of CMM, and it was also an unavoidable matter in the internationalised process of CMM. As a result, to establish a rational and scientifical standard about the administration times of CMM is not only an important supplementary of the traditional theory of TCM, but also a necessary path to realize modernization of them. In recent years, there were many researches about the *in vivo* process of CMM (Wang *et al*, 2010). However, most researchers mainly focused on the metabolism behavior of component in the body, identification of effective substance, and compatibility rule of compound recipe. Little attention was paid to the study on rational daily administration times, because there were still some shortages of the classical PK model: (1) Because of the multicomponents of CMM and their different metabolism characteristics, it is unsuitable to determine the administration times only by the PK parameters of some certain constituents; (2) Many medicines have obvious hystersis effects after administration and their $t_{1/2}$ values are very short, their effect might be maintained very strong even when the plasma concentration is lower than the lowest detectable limit (LDL). As a result, to determine the administration times of CMM through PK parameters may be lack of clinical significance, because the most important property of medicines is their clinical

therapeutic effect. As a result, it is imperative to establish a systemic, scientific, and practical theory which is more suitable for the research of administration times of CMM, e.g. the PD/PK model described in this paper.

In this study, we investigated the daily administration times of YCHD in the treatment of cholestatic jaundice in rats based on the model of PD/PK and we found that it was more appropriate to give YCHD once a day after a comprehensive analysis. It could be accepted that the method of PD/PK, which takes the effects as core and PK of some main components as assist, is more reliable and more closer to clinic. It could not only ensure the safety and effect in clinic, but also make up the shortages of the classical PK model. All these conclusions could provide some reference for clinical medication, meanwhile, the further clinical effect will remain to be evaluated in the future work.

In this research, we chose a number of different indicators for comprehensive evaluation of the PD of YCHD in rats. Though the results were accurate and reliable to some extent, the operation was time-consuming and there were many limitations when the PD indicators were not obvious after administration. Therefore, our group will continue to take PD/PK model as the work center, searching for endogenous substances (bio-markers) (Zhang *et al*, 2011) which could substitute the PD indicators and be susceptible enough to manifest the pathological state of body. The research work would be greatly simplified if the bio-markers are found.

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