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ORIGINAL ARTICLE

Effect of kanglaite on rat cytochrome P450

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Abstract

Context: Kanglaite (KLT) is an oily substance extracted from *Coix lacryma-jobi* Linn. (Cramineae) and has been proved to significantly improve the life span and quality of life of patients, when combined with chemotherapy, radiotherapy, or surgery.

Objective: The purpose of this study was to find out whether KLT influences the effect on rat cytochrome P450 (CYP) enzymes (CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP3A4) by using cocktail probe drugs *in vivo*.

Materials and methods: A cocktail solution at a dose of 5 mL/kg, which contained phenacetin (20 mg/kg), bupropion (20 mg/kg), tolbutamide (5 mg/kg), omeprazole (20 mg/kg), and midazolam (10 mg/kg), was given as oral administration to rats treated with 7 d intraperitoneal injection of KLT. Blood samples were collected at a series of time-points and the concentrations of probe drugs in plasma were determined by HPLC-MS/MS. The corresponding pharmacokinetic parameters were calculated by the software of DAS 2.0 (SPPS Inc., Chicago, IL).

Results: In the experiment, there was a statistically significant difference in the $t_{1/2}$, C_{max}, $AUC_{(0-\infty)}$, and CL for phenacetin, bupropion, tolbutamide, omeprazole, and midazolam. Our study showed that treatment with multiple doses of KLT had induction effect on rat CYP1A2, while CYP2B6, CYP2C9, CYP2C19, and CYP3A4 enzyme activities had been inhibited after multiple doses of KLT treatment.

Conclusions: KLT can either induce or inhibit activities of CYP. Therefore, caution is needed when KLT is co-administration with some CYP substrates in clinic, which may result in herb–drug interactions.

Keywords

CYP, interaction, KLT

History

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Introduction

Kanglaite (KLT) is an oily substance extracted from *Coix lacryma-jobi* Linn. (Cramineae). The main active ingredient is a compound of triglycerides containing four types of fatty acid (Yu et al., 2008). In clinical use in China, KLT has been proved to significantly improve the life span and quality of life of patients, when combined with chemotherapy, radiotherapy, or surgery (Pan et al., 2012; Shen et al., 2012; Zhan et al., 2012). Coix seed is traditional Chinese medicine that is well known for its antitumor and immunomodulatory effects. Evidence has accumulated concerning the medical use of coix seed and its extracts in the treatment of diseases such as cancer metastasis, hypertension, arthritis, asthma, and immunological disorders. Research shows that KLT principally blocks the G2 + M phase of the cell cycle, thereby reducing the mitotic division of cells and inhibiting the proliferation of tumor cells; at the same time, it can activate proapoptotic factors, leading to apoptosis (Lu et al., 2008). Although KLT injections have been successful in the treatment of various malignant tumors, it is unclear how this extract of coix seed acts on human cytochrome P450 (CYP) activities *in vivo*.

Herbal medicines are often used with western modern drugs with the aim to decrease side effects or toxicity, or to obtain a synergistic or additive effect in terms of pharmacological effects. For this reason, herbal medicine has received increasing popularity. However, it has been reported that herbal products containing a number of natural compounds can cause pharmacokinetic interaction with modern drugs when they were administrated simultaneously (Remirez et al., 2009).

Herbal products may alter the metabolism of certain drugs and, likewise, the metabolism of components of herbal products may be altered by some drugs, specifically those that affect CYPs. These can bring about an alteration of the pharmacokinetics and pharmacological activity of drug or herbal product components leading to adverse reactions following their co-administration (Danton et al., 2013; Goh et al., 2013). CYP is a superfamily of isozymes in which CYP1, CYP2, and CYP3 play major roles in the metabolism of many drugs (Pelkonen et al., 2008). The induction or inhibition of some of these isoforms can lead to clinically significant manifestations (Pelkonen et al., 1998). While drug candidates are routinely evaluated for their effect on drug-metabolizing enzymes during the development process, this is

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not the case for many herbal medicines. Recently, some herbal medicines were administered for the purpose of measuring the inhibition or induction of hepatic CYP enzymes. For example, andrographalide from *Andrographis paniculata* (Acanthaceae) was shown to alter CYP1A1 activity and mRNA expression in mice hepatic microsomes (Chatuphonprasert et al., 2009; Jarukamjorn et al., 2010). In addition, Yahom Ampanthong (combination of traditional medicines) inhibited CYP1A1, CYP1A2, and CYP2E1 activities in mice (Sirisangtrakul & Sripanidkulchai, 2011).

Nevertheless, there are no reports regarding the hepatic metabolism of KLT, neither the contribution of CYP enzymes to its metabolism nor the effect of the extract on various liver CYP isoforms. Therefore, the aim of this study was to investigate the effects of KLT on the *in vivo* activity of pivotal isoforms of rat hepatic CYP enzymes involved in drug metabolism including CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP3A4. This is expected to raise awareness of possible metabolic interactions with concomitant administered traditional and herbal medicines.

Materials and methods

Chemicals and reagents

KLT injection was purchased from Zhejiang Kanglaite Pharmaceutical Co., Ltd (Hangzhou, China). Phenacetin (purity > 98.0%), bupropion (purity > 98.0%), tolbutamide (purity > 98.0%), omeprazole (purity > 98.0%), midazolam (purity > 98.0%), and the internal standard carbamazepine (IS, purity > 98.0%) were also purchased from Sigma-Aldrich Company (St. Louis, MO). HPLC grade acetonitrile and methanol were from Merck Company (Darmstadt, Germany). All other chemicals were of analytical grade and used without further purification. Ultra-pure water (resistance > 18.2 mΩ) prepared by a Millipore Milli-Q purification system (Bedford, NY).

Apparatus

All analysis was performed with a 1200 Series liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a quaternary pump, a degasser, an autosampler, a thermostatted column compartment, and a Bruker Esquire HCT mass spectrometer (Bruker Technologies, Bremen, Germany) equipped with an electrospray ion source and controlled by ChemStation software (Agilent Technologies, Santa Clara, CA).

Animals

Male Sprague–Dawley rats with body weights of 220 ± 30 g were provided by the Animal Care and Use Committee of Wenzhou Medical College. They were housed in cages at 23–25 °C and allowed free access to regular rodent diet and water. After the 1-week acclimatization period, the rats were used for experiments and all efforts were made to minimize any animal suffering. All experimental procedures and protocols were reviewed and approved by the Animal Care and Use Committee of Wenzhou Medical College and were in accordance with the Guide for the Care and Use of Laboratory Animals.

Drug administration and sampling

Eighteen male Sprague–Dawley rats were randomly divided into three groups ($n = 6$): control group (CG), low-dose group (LG, 5.0 mL/kg), and high-dose group (HG, 10.0 mL/kg), which were given vehicle or KLT once daily. After intraperitoneal injection for consecutive 7 d, a cocktail solution at a dose of 5 mL/kg, which contained phenacetin (20 mg/kg), bupropion (20 mg/kg), tolbutamide (5 mg/kg), omeprazole (20 mg/kg), and midazolam (10 mg/kg) in CMC-Na solution, was administered orally to all rats in each group. Blood samples of each rat were collected pre-dose (0 h) and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 12 h after probe drugs administration through the tail vein and immediately separated by centrifugation at 8000 rpm for 10 min to obtain plasma. From the seventh blood collection, the rats were treated by oral administration of normal saline equal to the blood collection volume in order to restore blood capacity quickly. Plasma samples (100 μL) were transferred to another tube and stored frozen at −80 °C until analyzed.

Sample preparation

In a 1.5 mL centrifuge tube, aliquot of 0.2 mL acetonitrile with carbamazepine (500 ng/mL) as the internal standard was added to 0.1 mL of collected plasma sample. After the tube was vortex mixed for 1.0 min, the sample was centrifuged at 13 000 rmp for 10 min. Next, the supernatant (10 μL) was injected into the HPLC-MS/MS system for analysis.

Chromatographic conditions

Chromatographic separation was achieved on an Agilent Zorbax SB-C18 column (Agilent Technologies, Waldbronn, Germany) (150 mm × 2.1 mm, 3.5 μm) with the column temperature set at 30 °C. The mobile phase consisted of (A) acetonitrile and (B) 0.1% formic acid in water, and a gradient elution of 10–85% A at 0–1.5 min, 85–85% A at 1.5–6.0 min, 85–10% A at 6.0–7.0 min, and 10–10% A at 7.0–10.0 min was employed. The flow rate was 0.4 mL/min. The injection volume was 10 μL.

The quantification was performed by the peak-area method. The determination of target ions were performed in SIM mode (*m/z* 180 for phenacetin, *m/z* 240 for bupropion, *m/z* 271 for tolbutamide, *m/z* 362 for omeprazole, *m/z* 326 for midazolam, and *m/z* 237 for IS) and positive ion electrospray ionization interface. Drying gas flow was set to 6 L/min and temperature to 350 °C. Nebulizer pressure and capillary voltage of the system were adjusted to 20 psi and 3500 V, respectively.

Statistical analysis

The concentration–time profile of each probe drug was analyzed by DAS software (Version 3.0, Wenzhou Medical College, Zhejiang, China) and statistic analyses were tested by *t*-test using SPSS (Version 13.0, Wenzhou Medical College, Zhejiang, China). A value of $p < 0.05$ was considered to be statistically significant.

Results

A developed and validated HPLC-MS/MS method was used to determine the levels of the five probe drugs (phenacetin for CYP1A2, bupropion for CYP2B6, tolbutamide for CYP2C9, omeprazole for CYP2C19, and midazolam for CYP3A4) in rat plasma after intraperitoneal injection of KLT for 7 d.

Effect of KLT on the activity of CYP1A2 in rats

The effects of different treatment groups of KLT on pharmacokinetic parameters of phenacetin in rats are presented in Table 1. Mean plasma concentration–time curves of phenacetin in different groups are presented in Figure 1. After pretreatment with KLT, the $t_{1/2}$, T_{max}, C_{max}, $AUC_{(0-\infty)}$, and $MRT_{(0-\infty)}$ of phenacetin in LG were decreased significantly by 30.3, 29.4, 11.5, 42.8, and 23.3%, compared with those of CG, CL of phenacetin in LG was increased significantly by 37.3%. The $t_{1/2}$, T_{max}, C_{max}, $AUC_{(0-\infty)}$, and $MRT_{(0-\infty)}$ of phenacetin in HG were decreased significantly by 48.2, 40.5, 14.5, 65.2, and 73.4% compared with those of CG, CL of phenacetin in HG was increased significantly by 56.5%. The results indicated that metabolism of phenacetin in these treatment groups was evidently speeded up, and KLT had the potential to induce rat hepatic CYP1A2 activity *in vivo*.

Effect of KLT on the activity of CYP2B6 in rats

CYP2B6 activity was evaluated by comparing pharmacokinetic behaviors of bupropion in different groups. The pharmacokinetic profiles of bupropion before and after intraperitoneal injection of KLT for 7 d are shown in Table 2 and Figure 2. After pretreatment with KLT, the pharmacokinetic parameters (T_{max}, C_{max}, $AUC_{(0-\infty)}$, and $MRT_{(0-\infty)}$) of bupropion in LG were increased significantly by 34.6, 31.0, 33.9, and 34.0% compared with those of CG, CL of bupropion in LG was decreased significantly by 24.2%. The $t_{1/2}$, T_{max}, C_{max}, $AUC_{(0-\infty)}$, and $MRT_{(0-\infty)}$ of bupropion in HG were increased significantly by 62.9, 42.3, 78.2, 60.5, and 99.1% compared with those of CG, CL of bupropion in HG was decreased significantly by 40.4%. Our results indicated that KLT has an inhibitory effect on the activity of CYP2B6 after multiple intraperitoneal injection of KLT in rats.

Effect of KLT on the activity of CYP2C9 in rats

The effects of different treatment groups of KLT on pharmacokinetic parameters of tolbutamide in rats are presented in Table 3. Mean plasma concentration–time curves of tolbutamide in different groups are presented in Figure 3. After pretreatment with KLT, the $t_{1/2}$, T_{max}, $AUC_{(0-\infty)}$, and $MRT_{(0-\infty)}$ of tolbutamide in LG were increased significantly by 67.7, 39.1, 60.3, and 62.4% compared with those of CG, CL of tolbutamide in LG was decreased significantly by 43.9%. The $t_{1/2}$, T_{max}, C_{max}, $AUC_{(0-\infty)}$, and $MRT_{(0-\infty)}$ of tolbutamide in HG were increased significantly by 100.7, 58.1, 22.1, 148.4, and 94.3% compared with those of CG, CL of tolbutamide in HG was decreased significantly by 50.0%. The results indicated that metabolism of tolbutamide in these treatment groups was evidently slowed down, and KLT also had the potential to inhibit rat hepatic CYP2C9 activity *in vivo*.

Effect of KLT on the activity of CYP2C19 in rats

CYP2C19 activity was evaluated by comparing pharmacokinetic behaviors of omeprazole in different groups. The pharmacokinetic profiles and mean plasma concentration–time curves of omeprazole before and after oral administration of KLT for 7 d are shown in Table 4 and Figure 4. Compared with pre-administration, the T_{max}, C_{max}, and $AUC_{(0-\infty)}$ of omeprazole in LG were increased significantly by 11.0, 30.6, and 29.4% compared with those of CG, CL of omeprazole in LG was decreased significantly by 15.9%. No apparent influences were observed on other pharmacokinetic parameters in this treatment group. The $t_{1/2}$, T_{max}, C_{max}, $AUC_{(0-\infty)}$, and $MRT_{(0-\infty)}$ of omeprazole in HG were increased significantly by 26.6, 15.5, 51.2, 39.1, and 13.4% compared with those of CG, CL of omeprazole in HG was decreased significantly by 29.9%. According to the data, CYP2C19

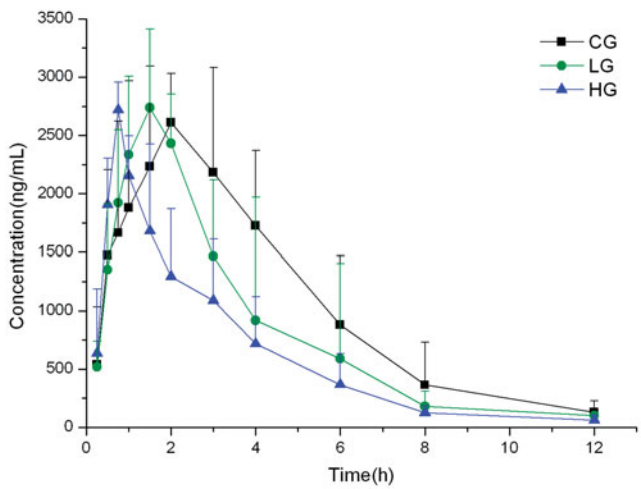

Figure 1. Mean plasma concentration–time curves of phenacetin in rats.

Table 1. Main pharmacokinetic parameters of phenacetin in rats ($n = 6$, mean ± SD).

Parameter	CG	LG	HG
$T_{1/2}$ (h)	2.948 ± 0.509	2.054 ± 0.656*	1.526 ± 0.515**
T_{max} (h)	1.700 ± 0.975	1.200 ± 1.006*	1.012 ± 0.354**
Cmax (ng/mL)	2984.818 ± 509.680	2640.417 ± 333.263*	2553.189 ± 185.213*
$AUC_{(0-\infty)}$ (μg·h/L)	13 058.122 ± 204.352	7474.209 ± 362.933**	4548.134 ± 377.698**
$MRT_{(0-\infty)}$ (h)	4.113 ± 1.456	3.156 ± 0.736*	1.095 ± 0.552**
CL (L/h/kg)	2.285 ± 0.552	3.138 ± 0.710**	4.575 ± 0.693**

*Significantly different from control, $p < 0.05$. **Significantly different from control, $p < 0.01$.

activity was inhibited by KLT after multiple intraperitoneal injections in rats.

Effect of KLT on the activity of CYP3A4 in rats

The effects of different treatment groups of KLT on pharmacokinetic parameters of midazolam in rats are presented in Table 5. Mean plasma concentration–time curves of midazolam in different groups are presented in Figure 5. After pretreatment with KLT, the T_{max}, C_{max}, $AUC_{(0-\infty)}$, and $MRT_{(0-\infty)}$ of midazolam in LG were increased by 21.2, 36.9, 48.8, and 55.9% compared with those of CG. The $t_{1/2}$, T_{max}, C_{max}, $AUC_{(0-\infty)}$, and $MRT_{(0-\infty)}$ of midazolam in HG were increased significantly by 49.9, 36.2, 55.4, 33.8, and 24.9% compared with those of CG, CL of midazolam in HG was decreased significantly by 27.5%. The results indicated that metabolism of midazolam in these treatment groups was evidently slowed down, and KLT had the potential to inhibit rat hepatic CYP3A4 activity *in vivo*.

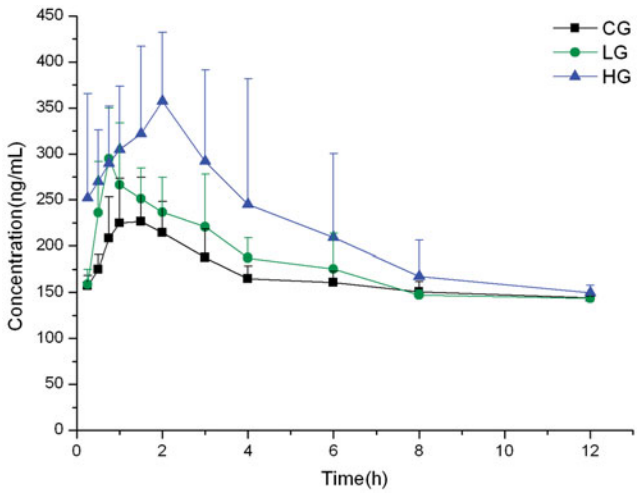

Figure 2. Mean plasma concentration–time curves of bupropion in rats.

Discussion

The concomitant administration of herbal supplements and synthetic drugs has become increasingly popular. Chinese herbal medicines contain a variety of biologically active ingredients. As a result, herb–drug interactions have become a common clinical problem (Izzo & Ernst, 2009). According to recent studies, the mechanisms underlying the interaction between herbal medicines and conventional drugs mainly involve induction or inhibition of the activities of metabolic enzymes and drug efflux proteins. Therefore, potential herb–drug interactions involving Chinese herbal medicines are worthy of study based on the CYP system (Izzo & Ernst, 2001).

KLT emulsion for infusion is prepared by extracting active antitumor components from the primary product of the Chinese plant Semen Coicis and formed as a lipid emulsion (Li, 2005). It is mainly used for the treatment of no-small cell lung cancer, liver cancer, gastric cancer, etc. (Pan et al., 2012; Shen et al., 2012; Zhan et al., 2012). The exact antitumor mechanism is unclear, although some studies showed that it may block tumor cell mitosis at the boundary of G2 and M phases of the cell cycle, induce tumor cell apoptosis, increase the expression of Fas/Apo-1 gene, reduce expression of bcl-2 gene, and selectively inhibit cyclooxygenase 2 (COX-2), not COX-1. Clinical data showed that KLT can decrease cancer cachexy and might reverse multiple drug resistance of tumor cells (Li, 2005). Given the high prevalence of use of traditional Chinese medicine among Chinese cancer patients, research is urgently needed to systematically evaluate the effect of KLT on CYP activities *in vivo*.

CYP1A2 accounts for about 13% of the total CYP content in human liver (Shimada et al., 1994) and is involved in the

Table 2. Main pharmacokinetic parameters of bupropion in rats ($n = 6$, mean ± SD).

Parameter	CG	LG	HG
$t_{1/2}$ (h)	25.528 ± 6.874	28.037 ± 5.713	41.587 ± 3.659**
T_{max} (h)	1.300 ± 0.570	1.750 ± 0.378**	1.850 ± 0.822**
C_{max} (ng/mL)	232.810 ± 45.536	305.025 ± 77.135**	414.846 ± 109.455**
$AUC_{(0-\infty)}$ (μg·h/L)	13 288.767 ± 2575.392	17 797.738 ± 3556.387**	21 321.860 ± 4819.019**
$MRT_{(0-\infty)}$ (h)	29.810 ± 7.850	39.954 ± 6.255*	59.359 ± 6.359**
CL (L/h/kg)	4.510 ± 0.630	3.419 ± 0.802*	2.683 ± 0.762**

*Significantly different from control, $p < 0.05$. **Significantly different from control, $p < 0.01$.

Table 3. Main pharmacokinetic parameters of tolbutamide in rats ($n = 6$, mean ± SD).

Parameter	CG	LG	HG
$t_{1/2}$ (h)	3.978 ± 1.063	6.670 ± 1.723**	7.982 ± 1.194**
T_{max} (h)	2.167 ± 0.408	3.014 ± 0.528**	3.425 ± 0.556**
Cmax (ng/mL)	12 090.859 ± 882.386	12 274.311 ± 671.091	14 759.583 ± 738.841*
$AUC_{(0-\infty)}$ (μg·h/L)	62 192.708 ± 971.129	99 714.429 ± 1056.162**	15 4497.559 ± 1360.837**
$MRT_{(0-\infty)}$ (h)	6.448 ± 1.051	10.473 ± 1.605**	12.529 ± 1.181**
CL (L/h/kg)	0.098 ± 0.043	0.055 ± 0.017*	0.049 ± 0.027**

*Significantly different from control, $p < 0.05$. ** Significantly different from control, $p < 0.01$.

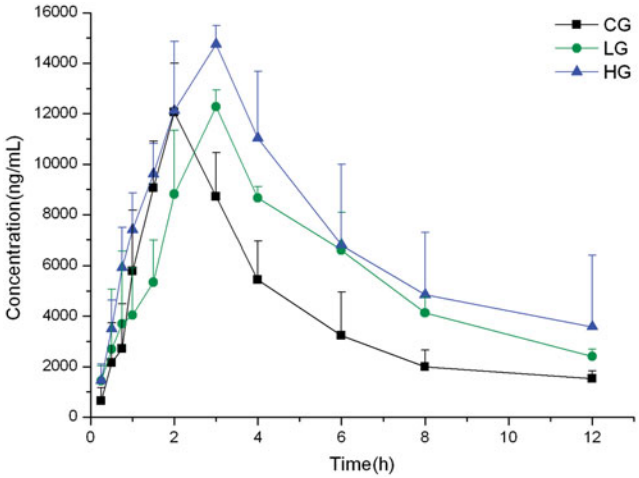

Figure 3. Mean plasma concentration–time curves of tolbutamide in rats.

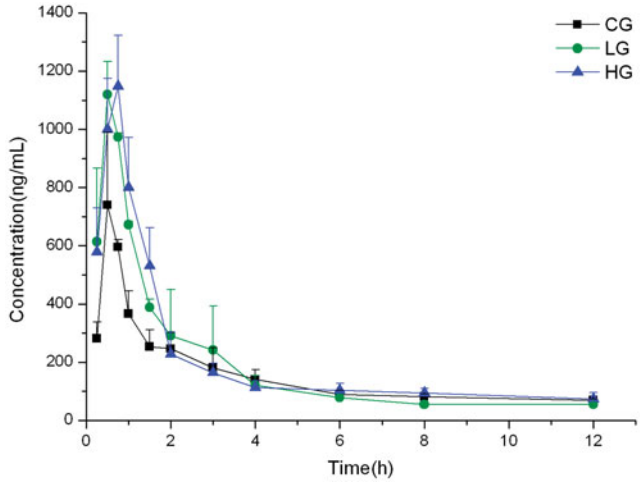

Figure 4. Mean plasma concentration–time curves of omeprazole in rats.

Table 4. Main pharmacokinetic parameters of omeprazole in rats ($n = 6$, mean ± SD).

Parameter	CG	LG	HG
$t_{1/2}$ (h)	10.763 ± 2.799	11.809 ± 3.478	13.630 ± 3.885*
T_{max} (h)	0.563 ± 0.125	0.625 ± 0.133*	0.650 ± 0.137*
C_{max} (ng/mL)	785.155 ± 170.782	1025.691 ± 124.746**	1187.134 ± 168.234**
$AUC_{(0-\infty)}$ (μg h/L)	2810.888 ± 180.021	3637.672 ± 185.953*	3908.607 ± 163.353**
$MRT_{(0-\infty)}$ (h)	12.933 ± 2.470	13.303 ± 2.679	14.660 ± 2.737*
CL (L/h/kg)	7.483 ± 1.046	6.293 ± 1.344*	5.242 ± 0.928*

*Significantly different from control, $p < 0.05$. ** Significantly different from control, $p < 0.01$.

Table 5. Main pharmacokinetic parameters of midazolam in rats ($n = 6$, mean ± SD).

Parameter	CG	LG	HG
$t_{1/2}$ (h)	2.922 ± 0.106	3.201 ± 0.872	4.380 ± 0.673**
T_{max} (h)	1.300 ± 0.274	1.650 ± 0.224*	1.770 ± 0.408**
C_{max} (ng/mL)	787.309 ± 134.767	998.509 ± 222.078*	1223.294 ± 287.794**
$AUC_{(0-\infty)}$ (μg·h/L)	5771.994 ± 328.676	6366.848 ± 188.644*	7722.623 ± 227.717**
$MRT_{(0-\infty)}$ (h)	5.753 ± 0.774	6.444 ± 0.685*	7.185 ± 0.143*
CL (L/h/kg)	1.875 ± 0.169	1.737 ± 0.199	1.359 ± 0.312**

*Significantly different from control, $p < 0.05$. ** Significantly different from control, $p < 0.01$.

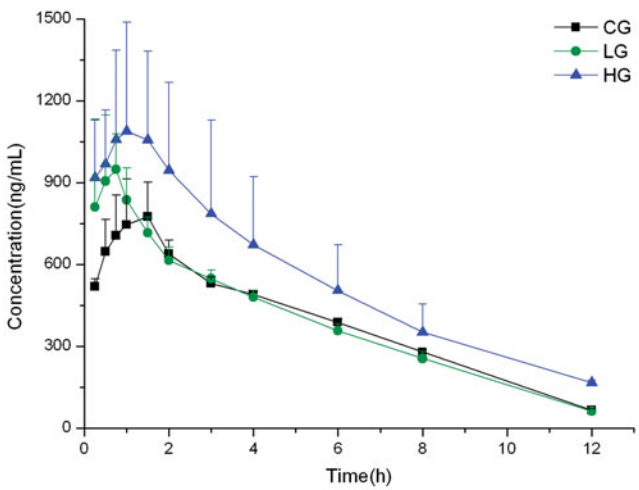

Figure 5. Mean plasma concentration–time curves of midazolam in rats.

metabolism of several endogenous compounds and some widely used drugs, also it could activate the procarcinogens such as aflatoxin B_1, a commonly recognized hepatocarcinogen (Mustajoki et al., 1994). In our study, we find that the inductive effect of KLT at high dosage on CYP1A2 was more potent after multiple dose administration for 7 d. It suggests that KLT has an inductive effect on the activity of CYP1A2 after a long period of abdominal injections in rats.

CYP2B6 is mainly expressed in the liver, accounting for 6% of total microsomal CYPs (Stresser & Kupfer, 1999), and various extrahepatic tissues, including the kidney, skin, brain, intestine, and lung (Gervot et al., 1999). CYP2B6 can metabolize ~8% of all pharmaceutical drugs, to some extent. These include cyclophosphamide, ifosfamide, tamoxifen, ketamine, artemisinin, and nevirapine (Zhou et al., 2009). According to our results, the inhibitory effect of KLT at high

dosage on CYP3A4 was more potent after long-period administration for 7 d. It inhibited CYP3A4 in a concentration-dependent manner, but the mechanisms underlying this effect are not yet known. Therefore, the dosage of KLT for treatment should be cautioned.

According to our results, investigation of CYP2C9 activity showed that as the administration dose increased, the effect of KLT on CYP2C9 was inhibition. As we know, CYP2C9 is one of the most abundant CYP enzymes in the human liver (~20% of hepatic total CYP content), where it metabolizes approximately 15% clinical drugs (>100 drugs), including a number of drugs with narrow therapeutic ranges (Miners & Birkett, 1998). The above results show that when KLT is used in combination with other drugs which and metabolized by the CYP2C9, the potential herb–drug interactions should be given more attention so as to reduce some adverse reactions or the failure in treatment due to low plasma concentration.

The second most abundant CYPs are members from the CYP2C subfamily (~20% of total hepatic CYP), accounting for approximately 16–20% of the CYP-mediated biotransformation (Gray et al., 1995). Members of this subfamily include CYP2C8, CYP2C9, CYP2C18, and CYP2C19 (Goldstein & De Morais, 1994) and the literature indicates that there are significant roles for CYP2C isoforms in human drug metabolism. Above all, CYP2C19 plays a significant role in the metabolism of the proton pump inhibitors and the anti-epileptic drugs diazepam and mephenytoin (Goldstein & de Morais, 1994). Therefore, the induction or inhibition on activity of CYP2C19 may lead to some undesirable effects. According to our results, CYP2C19 activity could be significantly inhibited by KLT after multiple doses administrations in rats. With the great use of KLT, people should pay more attention to its side effects caused by herb–drug interactions when they are administrated with other drugs, especially with substrates of CYP2C19.

CYP3A4 was known as the rate-limiting step in the metabolism and clearance of a large variety of clinical medications, including many pediatric drugs (Lu et al., 2003). In this study, we examined the activity of CYP3A4 by midazolam as a probe drug. Our results indicated that the inhibitory effect of KLT at high dosage on CYP3A4 was more potent after long-period administration for 7 d. It inhibited CYP3A4 in a concentration-dependent manner, but the mechanisms underlying this effect are not yet known.

Conclusions

In conclusion, the obvious effects of KLT *in vivo* as a probe of CYP1A2 metabolism suggest that there are clinically relevant herb–drug interactions between the drugs metabolized by this enzyme and KLT when they are used concomitantly. However, from our present results, we cannot exclude that co-medication of KLT with drugs metabolized by CYP2B6, CYP2C9, CYP2C19, and CYP3A4 may inhibit metabolism of these drugs and increase plasma concentrations of these drugs, which will result in relevant herb–drug interactions. Further clinical studies are required to fully assess the safety of KLT in terms of CYP.

Declaration of interest

The authors declare that they have no conflict of interests.

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