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Effect of colchicine on rat hepatic cytochrome P450 enzymes by cocktail probe drugs

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Colchicine (COL), an alkaloid derived from plants, has been used to treat gout, pseudogout and familial Mediterranean fever for several decades. The purpose of this study was to investigate the *in vivo* effect of COL on rat cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2C19 and CYP2D6) to assess its potential to interact with co-administered drugs. This was a randomized, double-blind, two-way crossover study with a 4-week washout period between the phases. Rats received COL *via* an irrigation stomach needle at a dose of 0.4 mg/kg once daily for consecutive 10 days. On the eleventh day, a cocktail solution at a dose of 4 ml/kg, which contained phenacetin (15.0 mg/kg), tolbutamide (3.0 mg/kg), omeprazole (15.0 mg/kg) and dextromethorphan (15.0 mg/kg), was oral administered to all rats. Then 0.3 ml blood samples were collected at a set of time-points. The plasma concentrations of probe drugs were simultaneously determined by HPLC-MS/MS. Pharmacokinetic parameters simulated by DAS software were used for the evaluation of COL on the activities of rat CYP1A2, CYP2C9, CYP2C19 and CYP2D6 enzymes. Our study showed that COL administration induced CYP2C9 activity, causing a significant decrease in $AUC_{(0\text{-}\infty)}$ ($P < 0.01$) and $t_{1/2}$ ($P < 0.05$) of tolbutamide, and a distinct increase in CL ($P < 0.01$). Many pharmacokinetic parameters of dextromethorphan in COL-treated rats were affected significantly, which indicated that the metabolism of dextromethorphan in these treatment groups was evidently slowed down. However, there was no significant influence of pharmacokinetic parameters of phenacetin and omeprazole in COL-treated rats. The results from the present *in vivo* study suggested that COL showed no effects on rat CYP1A2 and CYP2C19, however, it demonstrated potential inductive effects on CYP2C9 and inhibitory effects on CYP2D6. Therefore, caution is needed when COL is co-administered with drugs metabolized by CYP2C9 or CYP2D6, which may result in altered plasma concentrations of these drugs and relevant drug-drug interactions.

1. Introduction

The cytochrome P450 (CYP) superfamily, one of the most important drug-metabolizing enzyme systems in humans, is responsible for the oxidative metabolism of a vast variety of xenobiotics and endogenous compounds (Kim and Novak 2007). The most popular belief is that over 90% of drug metabolism in humans are mediated by CYPs. Inhibition or induction of any of the CYP isoforms has been recognized as the key role of unexpected and serious clinical drug-drug interactions (DDIs), especially when concomitant drugs are metabolized by the same enzyme (Michalets 1998; Tanaka and Hisawa 1999; Yan and Caldwell 2001). So, potential interaction is a major safety concern and may result in severe adverse reactions which are sometimes life-threatening. Due to the potential clinical outcomes of DDIs, there is a strong necessity to characterize, analyse and predict these interactions.

Colchicine (COL) is a natural product from the plant *Colchicum autumnale*. The therapeutic value of COL is well established in the treatment of acute gout flares (Ahern et al. 1987; Emmerson 1996; Neogi 2011; Terkeltaub 2003, 2010) and familial Mediterranean fever FMF) (Ben-Chetrit and Levy 1991; Dinarello et al. 1976). COL has also been suggested in the treatment of other inflammatory diseases such as Behcet disease (Hazen and Michel 1979), several vasculitides (Hazen and Michel 1979), and pericarditis (Adler et al. 1994). Multiple drug therapy is common therapeutic practice, particularly in patients with several diseases or in a complex state. Understanding DDIs on COL is important as many patients with gout are obese and have multiple comorbidities. This situation may induce a heightened potential for DDIs that increase COL drug exposure and subsequently increase the risk of COL toxicities (Akdag et al. 2006; Alayli et al. 2005; Bouquie et al. 2011; Caraco et al. 1992; Hsu et al. 2002; Hung et al. 2005; Rollot et al. 2004; van der Velden et al. 2008). In fact, the pharmacokinetics of COL have not been extensively characterized. Because of the lack of understanding of COL pharmacokinetics and DDIs, the effect of COL on the activity of the principal CYP enzymes involved in drug metabolism has become very important.

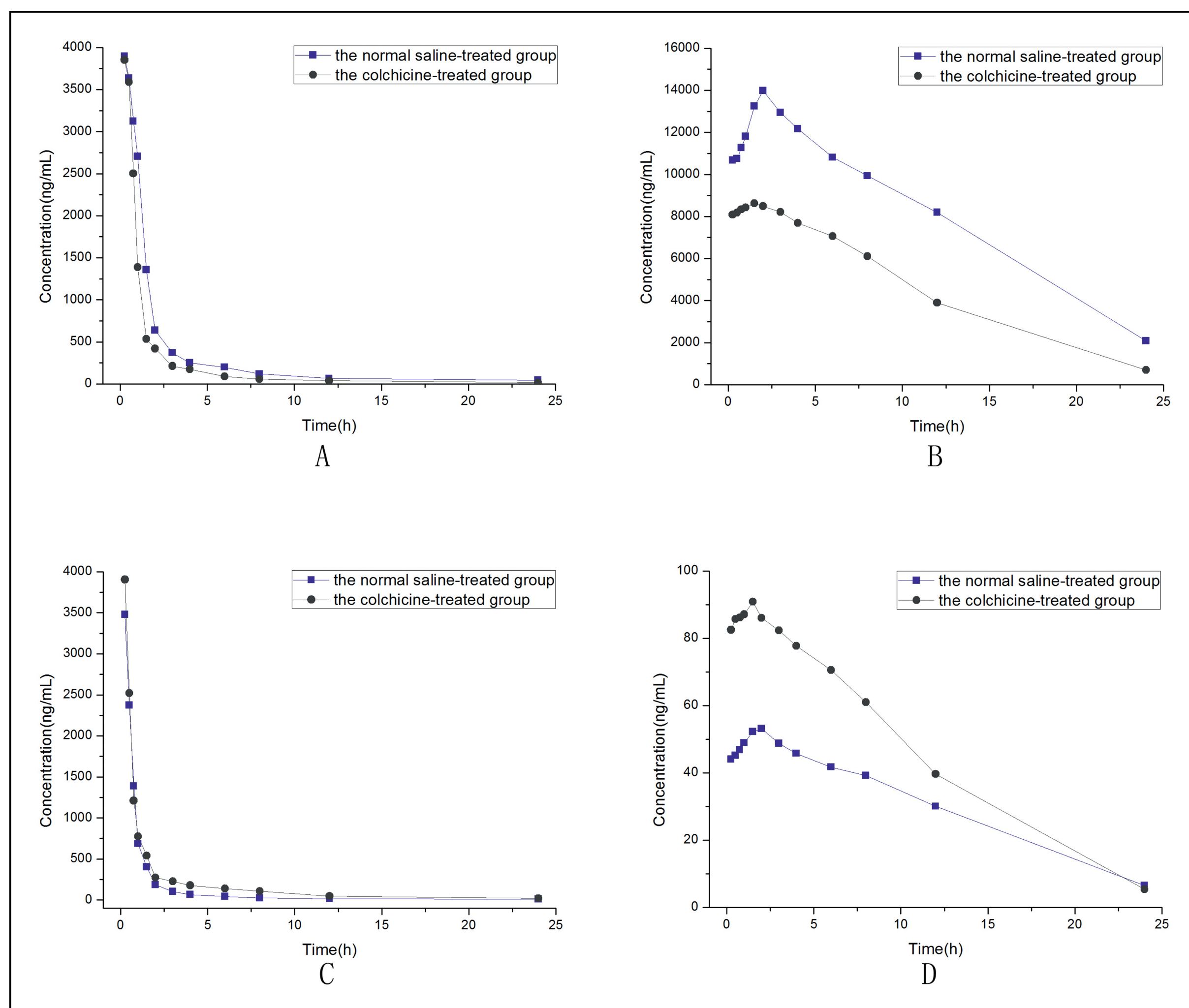

Fig.: Time-concentration curves of the four probe drugs after oral administration with COL 0.4 mg/kg in rats. A: Phenacetin; B: Tolbutamide; C: Omeprazole; D: Dextromethorpha.

Recently, a substrate "cocktail" method that uses a mixture of CYP-selective substrates and determines the substrates in a single assay using high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) has been developed (Ardjomand-Woelkart et al. 2011; Feidt et al. 2010; Flaherty et al. 2011; Zadoyan et al. 2011). The cocktail approach has several advantages; most conspicuously, multiple enzymes may be assessed in a single experiment, and the effect of intra- and inter-individual variability can be minimized (Breimer 1983; Tanaka et al. 2003). In the present study, we report an HPLC-MS/MS cocktail method consisting of phenacetin (PHE), tolbutamide (TB), omeprazole (OME), and dextromethorphan (DXM) to investigate the effects of COL on the CYP activities covering CYP1A2, CYP2C9, CYP2C19, CYP2D6 in rats.

2. Investigations and results

2.1. *Validation of the cocktail approach*

Under the conditions described in the experimental section, a reliable HPLC-MS/MS has been developed for simultaneous assessment of the activities of four CYP enzymes (CYP1A2, CYP2C9, CYP2C19 and CYP2D6) in rats. Standard curves of each probe drug in rat plasma were established over the following concentration ranges: 5.0–1000 ng/mL. The calibration curves were: $y = 0.00335c + 0.027775$ for PHE; $y = 0.0035\ c + 0.11405$ for TB; $y = 0.00395\ c + 0.06663$ for OME; $y = 0.023575\ c + 0.035475$ for DXM (c = concentration of each probe drug; y = peak area ratio of each probe drug versus internal standard). The results of linear regression analysis showed that the correlation coefficients of the calibration curves for all sample types were above 0.996. The detection limits of the assays for PHE, TB, OME and DXM were all found to be 5.0 ng/mL. The precision and accuracy of intra-day and inter-day of all the probe drugs for the low-, medium- and high-quality control samples were below 15%. The result of the chromatographic validation showed that the assay methods were suitable for this study.

2.2. *Effects of COL on rat CYP activities*

The main objective of this study was to evaluate the effect of COL on the pharmacokinetics of PHE, TB, OME and DXM. After COL oral treatment of rats with for 10 days, the pharmacokinetic parameters of the four probe drugs were simultaneously determined by HPLC-MS/MS. All pharmacokinetic analyses of the four probes were performed on pharmacokinetic data both for the control group (10 days of normal saline treatment) and the test group (10 days of COL treatment). The mean time-concentration curves of the four probe drugs were shown in the Fig. and pharmacokinetic parameters were shown in the Table. The values of the area under the plasma concentration-time curve from 0 time to infinity ($AUC_{(0\text{-}\infty)}$) were significantly smaller for TB those in COL-treated rats than that in normal saline-treated rats. The elimination half-time $t_{1/2}$ of TB was significantly shorter in the COL-treated group than in the normal saline-treated control group. And, the values of total clearance

Table: Effects of COL on pharmacokinetic parameters of the four probe drugs (mean ± SD, n = 14)

Probe Drug	Parameter	Normal saline	COL
	$AUC_{(0-\infty)}$(μg·h/L)	5128.932 ± 698.329	5045.722 ± 1306.760
	C_{max} (ng/mL)	3749.640 ± 935.553	3652.017 ± 1118.880
Phenacetin	$t_{1/2}$ (h)	8.975 ± 0.355	8.081 ± 3.632
	T_{max}(h)	0.293 ± 0.197	0.292 ± 0.102
	CL (L/h/kg)	3.041 ± 0.752	3.168 ± 0.929
	$AUC_{(0-\infty)}$(μg·h/L)	198733.286 ± 33635.258	113338.670 ± 10090.199[b]
	C_{max} (ng/mL)	14697.300 ± 1175.841	9347.448 ± 1712.081[b]
Tolbutamide	$t_{1/2}$ (h)	6.397 ± 1.297	5.229 ± 1.012[a]
	T_{max}(h)	2.200 ± 1.095	1.125 ± 0.586
	CL (L/h/kg)	0.015 ± 0.003	0.027 ± 0.002[b]
	$AUC_{(0-\infty)}$(μg·h/L)	3419.005 ± 257.810	4010.509 ± 296.883
	C_{max} (ng/mL)	3705.000 ± 978.538	3816.000 ± 1019.105
Omeprazole	$t_{1/2}$ (h)	5.508 ± 0.797	5.452 ± 0.909
	T_{max}(h)	0.286 ± 0.094	0.250 ± 0.086
	CL (L/h/kg)	3.404 ± 0.195	3.412 ± 0.224
	$AUC_{(0-\infty)}$(μg·h/L)	784.614 ± 125.755	1200.257 ± 235.622[b]
	C_{max} (ng/mL)	55.826 ± 3.523	87.137 ± 9.383[b]
Dextromethorphan	$t_{1/2}$ (h)	5.296 ± 1.921	6.510 ± 3.468[a]
	T_{max}(h)	1.607 ± 0.453	2.067 ± 0.376
	CL (L/h/kg)	19.590 ± 3.496	12.852 ± 2.197[b]

[a] $P < 0.05$; [b] $P < 0.01$ vs control

(CL) of TB in test rats were significantly larger than those in control rats. The results of TB suggested that COL could induce CYP2C9 in rats at a dose of 0.4 mg/kg every day for 10 days. Also, COL increased in elimination half-time $t_{1/2}$ and $AUC_{(0-\infty)}$ of DXM, and the corresponding decrease in CL demonstrated that COL could inhibit CYP2D6. The pharmacokinetics of PHE and OME showed no statistically significant difference from the normal saline-treated control rats. This means the activities of CYP1A2 and CYP2C19 are hardly altered by COL.

3. Discussion

COL is a tricyclic alkaloid ($C_{22}H_{25}NO$) which was isolated in 1820 and has a molecular mass of 399.437 (Ben-Chetrit and Levy 1998). Despite the widespread use of COL for the treatment of acute flares of gouty arthritis, familial Mediterranean fever (FMF), Behcet's disease, and recurring pericarditis with effusion, DDIs between COL and commonly used medications have not been extensively characterized. Analysis of the literature has revealed apparent safety issues when COL is administered indiscriminately or concurrently with CYP3A4 inhibitors or P-glycoprotein, possibly resulting in life-threatening conditions and an increased number of serious adverse events (Alayli et al. 2005; Bouquie et al. 2011; Hsu et al. 2002; Rollot et al. 2004). Considering the narrow therapeutic index and severely toxic side effects of COL, a potential pharmacokinetic interaction with CYP enzymes involved in drug metabolism is suggested.

Inhibition/induction of CYP activity by a medicine can significantly increase exposure of co-administrated drugs that are metabolised by the same CYP enzyme (Butterweck and Derendorf 2008; Lazarou et al. 1998). This can result in significant adverse events. Therefore, accurate models for predicting metabolic DDIs could be useful tools in order to avoid toxic adverse reactions. The cocktail approach has been used as a screening tool for potential *in vivo* DDIs, like the "Pittsburgh cocktail" (Frye et al. 1997) and "GW Cocktail" (Scott et al. 1999). Different HPLC-MS/MS methodologies have been developed for the fast and routine analysis of major *in vivo* and *in vitro* CYP activities simultaneously (Lahoz et al. 2008; Petsalo et al. 2008; Youdim and Saunders 2010). So, this approach was successfully used to investigate the effect of drugs on various CYP isoforms activities.

In our present study, the potential effect of COL on CYP activities including CYP1A2, CYP2C9, CYP2C19 and CYP2D6 in rats were assessed. According to our results, we find that COL increased the activity of CYP2C9, shortening the of TB. However, AUC(0-∞) ($P < 0.01$) and ($P < 0.05$) of DXM significantly increased, and the corresponding CL markedly decreased ($P < 0.01$), which illustrated that the activity of CYP2D6 tended to be inhibited. These complicated mechanisms of the effect of COL on the activities of CYP2C9 and CYP2D6 remain to be further studied. Meanwhile, we also found that COL did not influence the plasma concentration of PHE and OME as well as their associated pharmacokinetic parameters, and had very little or no affect on CYP1A2 and CYP2C19, respectively.

These regulating effects might have promising clinical significances, since CYP2C9 accounts for metabolism of approximately 15% clinical drugs, including a number of drugs with narrow therapeutic ranges (Zhou et al. 2009). TB has been well studied as marker of CYP2C9 activity and is most commonly used in CYP2C9 phenotyping studies (Wester et al. 2000). COL can increase the activity of CYP2C9, showing why COL improves liver metabolism of drugs, benefits the body by promoting excretion of endogenous substances and exogenous compounds, and also helps to explain the antitoxic mechanism. On the other hand, CYP2D6 is only a small percentage of all hepatic CYPs (< 2%), but it metabolizes ∼25% of all medications in the human liver (Cascorbi 2003). Thus, the inhibition of COL on CYP2D6 must be paid great attention on the account for changes in the pharmacokinetics of co-adminnitsered drugs, especially with substrates of CYP2D6. However, COL could not influence the activities of CYP1A2 and CYP2C19 which both are the major enzymes in the liver.

We conclude that it is of paramount clinical importance to avoid toxicity due to unanticipated decreased or increased COL exposure from concomitant use of CYP2C9 inducers or CYP2D6 inhibitors. Proper dose adjustments of COL will aid clinicians in the safe prescribing of COL. Avoidance of outdated high-dose COL regimens in the treatment of early acute gout flare,

especially in the presence of CYP2D6 inhibitors, provides an additional safety margin. When clinically appropriate, avoidance of the use of COL or the interacting drug is the best way to prevent DDIs.

4. Experimental

4.1. Chemicals and reagents

PHE, TB, OME, DXM and the internal standard carbamazepine (IS, all purity > 98.0%) were all purchased from Sigma-Aldrich Company (St. Louis, USA). HPLC-grade acetonitrile and methanol were from Merck Company (Darmstadt, Germany). All other chemicals were analytical grade and used without further purification. Ultra-pure water (resistance > 18.2 mΩ) prepared by a Millipore Milli-Q purification system (Bedford, USA) was used to make mobile phase and all other solutions.
Stock solutions of 1.0 mg/mL each of PHE, TB, OME, DXM and IS were prepared in methanol. The working standard solutions of each analyte were prepared by serial dilution of the stock solution with methanol. And IS was diluted to 500 ng/mL in acetonitrile as the precipitant.

4.2. Animals

Fourteen male Sprague-Dawley rats with body weights of 200 ± 20 g were provided by Wenzhou Medical College Laboratory Animal Center (Wenzhou, China). They were housed into house cages at 23–25 °C and had free access to regular rodent diet and water. After a 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.

4.3. Apparatus and Chromatographic Conditions

All analyses 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.
Chromatographic separation was achieved on a 150 mm × 2.1 mm, 3.5 µm particle, Agilent Zorbax SB-C18 column at 30 °C. A gradient elution programme was conducted for chromatographic separation with mobile phase A (0.1% formic acid in water) and mobile phase B (acetonitrile) as follows: 0–1.5 min (10–85% B), 1.5–6.0 min (85–85% B), 6.0–7.0 min (85–10% B), 7.0–10.0 min (10–10% B). The flow rate was 0.4 mL/min.
The determination of target ions were performed in SIM mode (m/z 180 for PHE, m/z 271 for TB, m/z 346 for OME, m/z 272 for DXM 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. Nebuliser pressure and capillary voltage of the system were adjusted to 20 psi and 3,500 V, respectively.

4.4. Sample preparation

An 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 in a 1.5 mL centrifuge tube. 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 for HPLC-MS/MS analysis.

4.5. Effects of COL on the CYP activities

This was a randomized, double-blind, two-way crossover study with a 4-week washout period between phases. In each phase 14 male SD rats were administered by gastric irrigation with normal saline or COL for 10 days. COL was administered orally at a dose of 0.2 mg/kg and normal saline was given in the same way. On the 11th day, three minutes after administration of COL, rats of the two groups were simultaneously administered an oral dose of 15, 3, 15 and 15 mg/kg of PHE, TB, OME and DXM, respectively. Blood samples of 0.3 mL were collected through the tail vein into heparinized 1.5 mL polythene tubes before (0 min) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 h after the administration of the probe drugs. The samples were immediately separated by centrifugation at 13,000 rpm for 10 min and 100 µL plasma samples were transferred to another tube and stored frozen at -80 °C until analyzed.

4.6. Statistical analysis

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

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