Effects of capsicine on rat cytochrome P450 isoforms CYP1A2, CYP2C19, and CYP3A4

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Abstract
Due to the frequent consumption of capsaicin (CAP) and its current therapeutic application, the correct assessment of this compound is important from a public health standpoint. The purpose of this study was to find out whether CAP affects rat cytochrome P450 (CYP) enzymes (CYP1A2, CYP2C19, and CYP3A4) by using cocktail probe drugs in vivo. A cocktail solution at a dose of 5 mL/kg, which contained phenacetin (15 mg/kg), omeprazole (15 mg/kg), and midazolam (10 mg/kg), was given orally to rats treated for 7 d with oral administration of CAP. Blood samples were collected at a series of time-points and the concentrations of probe drugs in plasma were determined by HPLC-MS. The results showed that treatment with multiple doses of CAP had no significant effect on rat CYP1A2. However, CAP had a significant inhibitory effect on CYP2C19 and an inductive effect on CYP3A4. Therefore, caution is needed when CAP is co-administered with some CYP substrates clinically because of potential drug–CAP interactions.

Introduction
For a long time, the hot pepper fruits that belong to the plant genus Capsicum (Solanaceae family) were among the most heavily consumed spices throughout the world. They were used as delicious spices with characteristic smells and tastes. The primary pungent ingredient in Capsicum fruits called capsicine (CAP) is responsible for the spiciness of pepper fruit1. The content of CAP in green and red peppers ranges from 0.1% to 1%2. CAP evokes numerous biological effects and thus has been the target of extensive investigations since its initial identification3,4. Many studies demonstrated that CAP promotes energy metabolism and suppresses accumulation of body fat5,6. Studies in humans confirm that CAP elevates body temperature and increases oxygen consumption7,8. CAP has been used for a number of diverse clinical conditions, such as chronic pain conditions, gastroprotection in non-steroidal anti-inflammatory drugs (NSAID) and ethanol use, post-operative nausea and vomiting, post-operative sore throat, and pruritus9. Moreover, there are several reports indicating that pretreatment with CAP might influence the activity of a category of drug metabolizing enzymes9,10,9,10,10,10,11. Many passive consumers of CAP take prescribed drugs concomitantly. This undoubtedly may raise the risk of drug–CAP interactions in these patients. Considering the frequent consumption of CAP and its current therapeutic application, correct assessment of this compound is important from the public health standpoint.

It has been reported that several cytochrome P450 (CYP) isozymes participate in the metabolism of CAP12. CYPs are oxidative enzymes that take part in the electron transfer system in the endoplasmic reticulum and are involved in the oxidative metabolism of numerous xenobiotics. Some CYP isozymes play important roles in the metabolism of medicinal compounds; therefore, unanticipated adverse reactions caused by drug–drug interactions mediated by CYPs have been reported13–15. Hence, when CAP is consumed, its potential effect on CYPs (and the metabolism of drugs being concurrently consumed) should be considered.

Evaluation of the effect of CAP on CYP enzyme activities is essential in clinical development as it may explain inter-subject variability, explicate potential toxic effects, and predict drug–CAP interactions. CYP-specific probe drugs can be used to determine the real time activities of important drug-metabolizing enzymes. Compared with the administration of single specific probe in multiple studies, the ‘‘cocktail’’ approach can monitor several CYP activities from several pathways in a single experiment16. Several different cocktails of markers have been used and many cocktail methods have been developed and evaluated in the past years. However, the disadvantages of this cocktail approach are also well defined: a possibility of probe-drug side-effects during in vivo use, an increased sample consumption, increased time consumption, and complicated analytical methods17. Nevertheless, the cocktail approach is widely used to assess the activities of CYP isoforms and is now one of the basic analytical tools in initial drug evaluation after developing precise analytical methods18.

For patients receiving multiple drug regimens for the treatment of different diseases, potential drug–CAP interactions may be important. Despite the popular use of CAP, the possibility of a
drug–CAP interaction when CAP is concomitantly administered with drugs that are metabolized by CYPs has not been investigated. The purpose of this study was to evaluate the effects of CAP on the metabolism of probe substrates of CYP isoforms (including phenacetin for CYP1A2, omeprazole for CYP2C19, and midazolam for CYP3A4).

Methods

Chemicals and reagents

CAP was obtained from Chengdu Must Bio-Technology Co. Ltd. (Chengdu, China; the purity no less than 98.0%). Phenacetin (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, MA) was used.

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 (Dayton, OH).

Animals

Male Sprague–Dawley rats with body weights of 220 ± 30 g were provided by the Animal Care and Use Committee of Wenzhou Medical University. The rats 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 University and were in accordance with the Guide for the Care and Use of Laboratory Animals.

Drug administration and sampling

Twelve male Sprague–Dawley rats were randomly divided into two groups (n = 6): the control group (CG) and the test group (TG, 30 mg/kg), which were given vehicle or CAP (dissolved in 0.5% CMC-Na solution) once daily. After oral administration for 7 consecutive days, a cocktail solution at a dose of 5 mL/kg, which contained phenacetin (15 mg/kg), omeprazole (15 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 0.17, 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, and 24 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 of the same blood collection volume in order to restore blood capacity quickly. 100 μL plasma samples were transferred to another tube and stored frozen at −80 °C until analyzed.

Sample preparation

In a 1.5 mL centrifuge tube, a 0.2 mL aliquot of acetonitrile with carbamazepine (100 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 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 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. Nebuliser 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 University, Wenzhou, China) and statistical significance was tested by a two-tailed t-test using SPSS (Version 13.0, Wenzhou Medical University, Wenzhou, China). A value of p < 0.05 was considered to be statistically significant.

Results

In this study, an HPLC-MS method was developed and validated and used to determine the levels of the three probe drugs (phenacetin for CYP1A2, omeprazole for CYP2C19, and midazolam for CYP3A4) in rat plasma after oral administration of CAP for 7 d.

Effect of CAP on the activity of CYP1A2 in rats

The pharmacokinetic profile of phenacetin after CAP treatment was used to describe the activity of CYP1A2. The effect of CAP on pharmacokinetic parameters of phenacetin in rats is presented in Table 1. Mean plasma concentration–time curves of phenacetin in rats is presented in Figure 1. Compared with CG, the pharmacokinetic parameters (t1/2, Tmax, Cmax, AUC(0–∞), MRT(0–∞), and CL) of phenacetin showed no significant change. Our results indicated that CAP had no inductive or inhibitory effect on the activity of CYP1A2 after multiple oral administrations in rats.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CG</th>
<th>TG</th>
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<tr>
<td>t1/2 (h)</td>
<td>9.217 ± 3.170</td>
<td>9.510 ± 3.331</td>
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<tr>
<td>Tmax (h)</td>
<td>0.157 ± 0.011</td>
<td>0.160 ± 0.014</td>
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<tr>
<td>Cmax (mg/mL)</td>
<td>385.382 ± 71.085</td>
<td>392.695 ± 60.064</td>
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<tr>
<td>AUC(0–∞) (μg·h/L)</td>
<td>1756.343 ± 228.433</td>
<td>1905.951 ± 366.167</td>
</tr>
<tr>
<td>MRT(0–∞) (h)</td>
<td>9.974 ± 3.470</td>
<td>10.015 ± 3.650</td>
</tr>
<tr>
<td>CL (L/h/kg)</td>
<td>5.775 ± 0.758</td>
<td>5.625 ± 0.691</td>
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</table>
Effect of CAP on the activity of CYP2C19 in rats

CYP2C19 activity was evaluated by comparing pharmacokinetic behaviors of omeprazole between CG and TG. The pharmacokinetic profiles and mean plasma concentration–time curves of omeprazole before and after oral administration of CAP for 7 d are shown in Table 2 and Figure 2. Compared with the pre-administration, the $t_{1/2}$ of omeprazole increased from 7.783 h to 9.809 h, the $T_{\text{max}}$ increased from 0.164 h to 0.185 h, the $C_{\text{max}}$ increased to 1.093 times of CG, the AUC(0–1) significantly increased (1.384 times of CG), and the CL significantly decreased. It was seen that CYP2C19 activity was significantly inhibited by CAP after multiple oral administrations in rats.

Effect of CAP on the activity of CYP3A4 in rats

As shown in Table 3 and Figure 3, compared with CG, the $t_{1/2}$ of midazolam was significantly decreased from 7.974 h to 6.877 h, the $T_{\text{max}}$ changed little, the $C_{\text{max}}$ decreased to 0.829 times of CG, the AUC(0–1) was significantly decreased (0.785 times of CG), and the CL significantly increased by 27.1%. These results suggested that CYP3A4 activity was significantly induced by CAP after multiple oral administrations in rats.

Discussion

Our results showed that treatment with multiple doses of CAP had no significant effect on rat CYP1A2. However, CAP had a significant inhibitory effect on CYP2C19 and an inductive effect on CYP3A4 after multiple doses of CAP treatment.

CYPs constitute a superfamily of heme-containing mono-oxygenases that catalyze the oxidative metabolism of a wide variety of xenobiotics, including drugs, plant-derived or fungal-derived secondary metabolites consumed with food, and a large number of environmental pollutants. The human CYP isoforms that metabolize xenobiotics belong to the families CYP1, CYP2, and CYP3. Individual CYP enzymes in these families have broad and overlapping substrate specificities, and are responsible for the metabolism of approximately 70–80% of all currently used drugs.

CYPs represent the major site for drug–drug and herb–drug interactions. Inhibition of CYPs can lead to clinically relevant increases in the exposure of the affected drug and thus an
increased toxicity. Further, some of the CYP isoforms are also subject to induction by xenobiotics via activation of nuclear receptors, resulting in a decreased exposure of the affected compound, and thereby leading to therapeutic failure or toxicological implications due to higher levels of a toxic metabolite. Moreover, many carcinogenic xenobiotics are metabolized by CYPs to either biologically inactive metabolites or chemically reactive electrophilic metabolites that covalently bind to DNA resulting in carcinogenicity. Since many chemical carcinogens are metabolized by CYPs to both inactive and active carcinogenic metabolites, the inductive effects of CAP on these enzymes will depend on the inductive effect on different metabolic pathways. A number of natural products have been demonstrated to modulate CYPs, including the induction of specific CYP isoforms, and the activation or inhibition of these enzymes. The majority of serious cases of drug interactions are as a result of the interference of the metabolic clearance of one drug by yet another co-administered drug, food or natural product. For a new molecular entity, it is important to assess its possible inhibitory or inductive effects on CYP enzymes.

It is well known that a major contributing factor of the drug–drug interaction is the inhibition of CYP enzyme-mediated activities, of which human CYP1A2 accounts for about 13% of the total CYP content in human liver. CYP1A2 is mainly responsible for metabolizing a variety of clinically important drugs, such as clozapine, ropivacaine, olanzapine, and theophylline. In addition to this, CYP1A2 also metabolizes a number of procarcinogens and endogenous substrates. In our study, we found that there was no significant difference of the pharmacokinetic parameters of the phenacetin before and after the administration of CAP. It suggested that CAP had no inhibitory or inductive effect on the activity of CYP1A2 after multiple oral administrations in rats.

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. Members of this subfamily include CYP2C8, CYP2C9, CYP2C18, and CYP2C19. 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 memantine. 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 CAP after multiple oral administrations in rats. With the great use of CAP as a food additive, it is important to mind the side effects caused by CAP when they are administrated with other drugs, especially with substrates of CYP2C19.

The modulating effects described in this work may have promising clinical consequences, since CYP3A4 represents about 30–70% of the total CYP in most human liver and intestines, respectively. The inhibition on CYP3A could be important for many clinical conditions, such as the endogenous metabolism, specific physiological functions, the biotransformation of ubiquitous environmental pollutants, and changes in the pharmacokinetics of co-administered drugs. CYP3A4 is the major isoenzyme in the liver. According to our results, CAP can increase the activity of CYP3A4. This may be one reason why CAP helps to restore hepatic drug metabolism, helps the body discharge exogenous materials and products, and may help to explain the antitoxic mechanism of CAP itself. The above results evidence that when CAP is used in combination with other drugs which metabolized by CYP3A4, the potential drug–CAP interactions should be carefully noted, as to reduce some adverse reactions in treatment due to low plasma concentration.

Conclusion

In conclusion, the inconspicuous effect of CAP in vivo on probe of CYP1A2 metabolism suggests that there are no clinically relevant drug–CAP interactions between the drugs metabolized by this enzyme and CAP when they are used concomitantly. However, from our present results, we could not exclude that comedication of CAP with drugs metabolized by human CYP2C19 and CYP3A4 may inhibit or induce metabolism of these drugs and change plasma concentrations of these drugs, which will result in relevant drug–CAP interactions. Further clinical studies are required to fully assess the safety of CAP in terms of CYP.

Declaration of interest

The authors report that they have no conflicts of interest.

References