Effects of curcumin on the pharmacokinetics of amlodipine in rats and its potential mechanism

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ABSTRACT

Context: Hyperlipidaemia and hypertension are often treated together with curcumin and amlodipine. It is necessary to investigate the drug-drug interaction between curcumin and amlodipine.

Objective: The interaction between curcumin and amlodipine was investigated in rats and with rat liver microsomes.

Methods: The pharmacokinetics of amlodipine (1 mg/kg) was investigated in rats with or without curcumin pre-treatment (2 mg/kg), six rats in each group. The metabolic stability of amlodipine was investigated with rat liver microsomes.

Results: Curcumin significantly increased the $C_{\text{max}}$ (26.19 ± 2.21 versus 17.80 ± 1.56 μg/L), $AUC_{(0-t)}$ (507.27 ± 60.23 versus 238.68 ± 45.59 μg·h/L), and $t_{1/2}$ (14.69 ± 1.64 versus 11.43 ± 1.20 h) of amlodipine ($p < 0.05$). The metabolic stability of amlodipine was significantly increased with the half-life time in rat liver microsomes increased from 34.23 ± 3.33 to 44.15 ± 4.12 min, and the intrinsic rate decreased from 40.49 ± 3.26 to 31.39 ± 2.78 μmol/min/mg protein.

Discussion and conclusions: These results indicated that drug-drug interaction might appear during the co-administration of curcumin and amlodipine. The potential mechanism may be due to the inhibition of CYP3A4 by curcumin. Thus, this interaction should be given special attention in the clinic and needs further experiments to characterize the effect in humans.

Introduction

Curcumin is a kind of polyphenol isolated from Curcuma Longae Rhizoma Purveratum, which is usually used as a food colour, but is also used in the clinic for the treatment and prevention of various diseases (Goel et al. 2008). Previously, curcumin was reported to possess a number of pharmacological effects, including antitumor, antioxidant, and anti-inflammatory (Chainoglou and Hadjipavlou-Litina 2019). For example, curcumin can inhibit the growth and invasion of human monocytic leukemia SHI-1 cells by regulating MAPK and MMP signalling (Zhu et al. 2020), it also can inhibit the progression of breast cancer (Mittal et al. 2020; Pereira et al. 2020). In the clinic, curcumin is always used for the therapy of hyperlipidaemia, which makes it easier to combine with other drugs (Zingg et al. 2013; Panahi et al. 2018).

As hyperlipidaemia and hypertension have many common risk factors and have close associations in many aspects, the clinical therapy of hyperlipidaemia and hypertension always combines different drugs to make the therapy more effective (Ruixing et al. 2009). Amlodipine is one of the most common drugs used for the treatment of hypertension, which is usually co-administered with other hypolipidemic drugs, such as simvastatin (Fuhrmann et al. 2019). Co-administration of different drugs could induce drug-drug interactions, which can affect the pharmacokinetics of drugs. For example, epigallocatechin-3-gallate can inhibit the metabolism of amlodipine due to the inhibitory effect on the activity of CYP3A4 (Han et al. 2019). Curcumin was demonstrated to be an inhibitor of CYP3A4, which is responsible for the metabolism of amlodipine (Lee et al. 2011; Zhu et al. 2014). Moreover, curcumin can be combined with amlodipine for the treatment of hyperlipidaemia and hypertension. Therefore, it is necessary to investigate the drug–drug interaction between curcumin and amlodipine, which can directly affect the pharmacokinetic and pharmacological effect of amlodipine.

In this study, the drug–drug interaction between curcumin and amlodipine was studied in rats, to explore the effect of curcumin on the pharmacokinetics of amlodipine and its potential mechanism, which can provide more guidance for the clinical co-administration of curcumin and amlodipine.

Materials and methods

Materials and reagents

Standards of amlodipine (purity >98%), felodipine (purity >98%) and curcumin (purity >98%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). β-NADPH was obtained...
from Sigma Chemical Co. (St. Louis, MO). Pooled rat liver microsomes (RLM) were purchased from BD Biosciences Discovery Labware (Woburn, MA). Acetonitrile and methanol were purchased from Fisher Scientific (Fair Lawn, NJ). Formic acid was purchased from Anaqua Chemicals Supply Inc. Limited (Houston, TX). Ultrapure water was prepared with a Milli-Q water purification system (Millipore, Billerica, MA). All other chemicals were of analytical grade or better.

**Animals**
Male Sprague–Dawley rats weighing 230–250 g were supplied by Sino-British Sippr/BK Lab Animal Ltd (Shanghai, China). Animals were sustained under 22 ± 2 °C and 50 ± 10% relative humidity in an air-conditioned animal quarter, and acclimatized to the facilities for 5 days. Water and food (laboratory rodent chow, Shanghai, China) were allowed *ad libitum*. Before each experiment, animals were fasted with free access to water for 12 h. The experiment was approved by the Animal Care and Use Committee of Yidu Central Hospital of Weifang.

**LC-MS/MS and conditions**
The concentration of amlodipine was analyzed using an Agilent 1290 series liquid chromatography system (Agilent Technologies, Palo Alto, CA) as previously reported (Zhang et al. 2018). Briefly, the sample was separated on Waters Xbridge C18 column (100 × 3.0 mm, i.d.; 3.0 μm, Waters Corporation, Milford, MA) and eluted with an isocratic mobile phase: solvent A (water containing 0.1% formic acid) – solvent B (acetonitrile) (65:35, v/v). The column temperature was set at 25 °C, the flowing rate at 0.4 mL/min and the injection volume at 5 μL. Mass spectrometric detection was carried out on an Agilent 6460 triple-quadruple mass spectrometer (Agilent Technologies, Santa Clara, CA) with Turbo Ion spray, which is connected to the liquid chromatography system. The mass scan mode was MRM positive. The precursor ion and product ion were *m/z* 409.2 → 206.1 for amlodipine, and *m/z* 384.5 → 338.1 for felodipine as the internal standard. The collision energy for amlodipine and felodipine was 30 and 20 eV, respectively. The MS/MS conditions were optimized as follows: fragmentor, 110 V; capillary voltage, 3.5 kV; nozzle voltage, 500 V; nebulizer gas pressure (N2), 40 psig; drying gas flow (N2), 10 L/min; gas temperature, 350 °C; sheath gas temperature, 400 °C; sheath gas flow, 11 L/min. Agilent MassHunter B.07 software was used for the control of the equipment and data acquisition. Agilent Quantitative analysis software (Agilent Technologies, Santa Clara, CA) was used for data analysis.

**Pharmacokinetic experiment**
The pharmacokinetics of amlodipine was investigated in rats. Twelve rats were divided into two groups randomly, including group A: pre-treated with curcumin and group B: without pre-treatment of curcumin. The amlodipine and curcumin fraction powders were all homogenized in 1.5% Tween 80 aqueous solution with a mortar and pestle. For group A, curcumin was administrated to rats at a dose of 2 mg/kg for 10 days. The dose of amlodipine administrated to both group A and group B was 1 mg/kg. After the administration of amlodipine 0, 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, and 48 h, the plasma samples were collected into a heparinized tube via the oculi chorioideae vein. Plasma samples were centrifuged at 3500 rpm for 10 min, and the supernatant was stored at ~80 °C until analysis.

**Preparation of rat plasma samples**
To 100 μL aliquot of a plasma sample, 20 μL methanol and 180 μL internal standard methanol solution (2 ng/mL) were added and vortexed for 60 s to mix in a 1.5 mL polypropylene tube, and were centrifuged at 12,000 rpm for 10 min. The supernatant was removed into an injection vial, and a 3 μL aliquot was injected into the LC-MS/MS system for analysis.

**Metabolic experiment with rat liver microsomes**
Using rat liver microsomes, the metabolic stability of amlodipine was investigated. The reaction mixture was incubated at 37 °C for 5 min and then amlodipine (1 μM) was added. For the curcumin, curcumin was added into rat liver microsomes before the addition of amlodipine, and preincubating for 30 min at 37 °C. After incubating for 0, 1, 3, 5, 15, 30, and 60 min, aliquots of 30 μL were collected from reaction volumes. Ice-cold acetonitrile (60 μL) containing felodipine was used to terminate the reaction. The sample preparation method was the same as the plasma sample preparation method. The concentration of amlodipine was detected by LC-MS/MS.

The metabolic stability of amlodipine was evaluated by the value of half-life (*t*1/2) *in vitro*, calculated by the following equations:

\[
 t_{1/2} = 0.693/k; \\
 V \ (\mu L/mg) = \frac{\text{volume of incubation (μL)}}{\text{protein in the incubation (mg)}}; \\
 \text{Intrinsic clearance (Clint) (μL/min/mg protein)} = V \times 0.693/t_{1/2}.
\]

**Statistical analysis**
All data were presented as mean value ± SD of three experiments. The pharmacokinetic parameters were calculated using DAS 3.0 pharmacokinetic software (Chinese Pharmacological Association, Anhui, China). Differences between groups were analyzed by one-way ANOVA, and it was considered to be statistical when *p* < 0.05.

**Results**
Curcumin increased the system exposure of amlodipine

Figure 1 shows the pharmacokinetic profile of amlodipine with or without pre-treated with curcumin. The administration of curcumin significantly affected the pharmacokinetic profile, as the peak plasma concentration (*C* max) and the time reach *C* max was significantly different.

Corresponding pharmacokinetic parameters are summarized in Table 1. The *C* max of amlodipine is 17.80 ± 1.56 μg/L, which was significantly increased to 26.19 ± 2.21 μg/L after pre-treatment with curcumin (*p* < 0.05). Meanwhile, curcumin also increased the *AU*C0–1 of amlodipine from 238.68 ± 45.59 to 507.27 ± 60.23 μg/h/L significantly (*p* < 0.05). The half-life time (*t*1/2) and the *T* max of amlodipine were prolonged by the administration of curcumin, which is consistent with the decrease of
common drugs for the treatment of hyperlipidaemia and hyper-
blood pressure, these two diseases are often treated together with com-
mon risk factors and have close associations in many
treatment of similar diseases. Hyperlipidaemia and have many
different kinds of drugs is widely accepted, especially for the

With the development of medical science, co-administration of

together in the clinic. Drug–drug interaction is the main
factor that affects the metabolism of drugs, which is closely asso-
ciated with the pharmaceutical effect or toxicity of drugs. For
example, co-administration of triptolide and fenofibrate induces
drug–drug interaction, which inhibits the metabolism of fenofi-
bcrate (Li et al. 2019).

In this study, we found the pharmacokinetic profile of amlo-
dipine was affected, as the AUC(0–t), t1/2, and other pharmacoki-
etic parameters were changed after combining with curcumin.
These results indicated that curcumin inhibited the metabolism
of amiodipine. Furthermore, the intrinsic clearance rate of amlo-
dipine in rat liver microsomes was decreased by curcumin, which
suggested an increase in the metabolic stability of amiodipine.
Previously, inhibition or induction of cytochrome P450 enzymes
(CYP450) was reported to be the main cause responsible for the
drug–drug interaction. Especially, the activity of CYP3A4, an
enzyme in the liver involved in the metabolism of various drugs,
plays vital roles during the pharmacokinetics of several drugs,
such as puerarin, warfarin, and asiasian acid, where the inhibition
on CYP3A4 resulted in the drug–drug interaction (Guo et al.
2018; Liu et al. 2019; Song et al. 2020). It has been reported that
amiodipine is the substrate of CYP3A4, and curcumin has been
demonstrated to be an inhibitor of CYP3A4 (Lee et al. 2011;
Zhu et al. 2014). Therefore, we speculated the effect of curcumin
on the pharmacokinetics of amiodipine was a result of the inhib-
ition of CYP3A4. Additionally, similar drug–drug interaction
may occur between amiodipine and drugs that affect the activity
of CYP3A4 or curcumin and drugs metabolized by CYP3A4.
These potential interactions need more in vivo or in vitro experi-
ments to identify.

However, this is an in vivo study in rat provided evidence for
the potential drug–drug interaction between curcumin and amlo-
dipine, which cannot represent the interaction in human.
Therefore, more experiments that more closely resemble human
metabolism are needed in future studies.

Conclusions
Co-administration of curcumin and amiodipine may induce
drug–drug interaction, which increases the system exposure of
amiodipine due to the inhibition of CYP3A4. Therefore, the
combination of these two drugs should be given special attention
in the clinic. There are a number of drugs that affect the activity
of CYP3A4 or curcumin and drugs metabolized by CYP3A4.
Potential drug–drug interactions might appear during co-administration, which needs further
studies to verify.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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