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Pharmacokinetic based study on "lagged stimulation" of *Curcumae Longae Rhizoma - Piper nigrum* couplet in their main active components' metabolism using UPLC-MS-MS

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ABSTRACT

Background: *Curcumae Longae Rhizoma* is one of the commonly used traditional Chinese medicines, which has multiple biological activities such as relieving stagnation and stasis, pain alleviation, curing amenorrhea and wounds. However, its main active component-curcumin has poor absorption and very fast metabolism in body. To solve this problem, *Piper nigrum* was introduced for its ability to strengthen bioavailability of other compounds.

Purpose: In most cases of TCM couplets, all ingredients were prepared and taken simultaneously, which in our opinion did not take full advantage of their interactions. Therefore, order of administration should be adjusted according to pharmacokinetic parameters of the ingredients, which the ones act as supplement can first be taken, and main therapeutic components followed when the former reached its peak.

Method: the extract of *Piper nigrum* (containing at least 95% piperine) was taken by rats 6 h before taking *Curcumae Longae Rhizoma* extract (containing at least 95% curcumin). Then, a UPLC-MS-MS method was developed to determine their content in plasma simultaneously. Determination was carried out by on a C18 column within 5 min by isocratic elution using 0.2% formic acid and acetonitrile (50:50, v/v). Tandem mass detection was conducted by selective reaction monitoring (SRM) via electrospray ionization (ESI) source in positive mode. Samples were pre-treated by liquid-liquid extraction (LLE), and verapamil was used as internal standard (IS).

Results: For both curcumin and piperine, the proposed method had good linearity ($r^2 = 0.999$) within the concentration range of 1–1000 ng/ml, with good recovery, precision and stability. The lower limit of quantification (LLOQ) was 1 ng/ml. As pharmacokinetic data indicated, Maximum concentration (C_{max}) of curcumin increased significantly to 394.06; the time reach maximum concentration (T_{max}) and elimination half-life ($T_{1/2}$) were 0.5 and 0.67 h, respectively;

Conclusion: The results provide a good strategy for the investigation of TCM formula especially the couplets, as well as a fast, selective and sensitive UPLC-MS-MS method determining active components in-vivo. Furthermore, the finding of "lagged stimulation" suggested that the use of complex formula should take pharmacokinetics into much more careful consideration.

Introduction

Curcumae Longae Rhizoma (or jianghuang in Chinese) is an herbal ingredient derived from dried roots of *Curcuma longa L*, (The Pharmacopoeia Commission of People's Republic of China, 2015a,b), according to official record, it traditionally used for relieving stagnation, stasis and pain, as well as curing amenorrhea and wounds (The Pharmacopoeia Commission of People's Republic of China, 2015a,b). All plant names were checked with http://www.theplantlist.org. Pharmacological research showed

Abbreviations: TCM, Traditional Chinese Medicine; Cur, curcumin; Pip, piperine; UPLC-MS-MS, ultra-performance liquid chromatography–mass spectrometry; ESI, electrospray ionization; LLOQ, lower limit of quantification; C_{max}, maximum concentration; T_{max}, the time reach maximum concentration; $T_{1/2}$, elimination half-life.

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that *Curcumae Longae Rhizoma* also has the effect of antioxidant, anti-inflammatory, anti-thrombotic, hepatoprotector (Naika et al., 2004), and anti-hyperlipidemia (Fan et al., 2006; Babu and Srinivasan, 1997; Soni and Kuttan, 1992; Soudamini et al., 1992), which is correspondent to its indication of promoting qi and digesting bruise in TCM theory (Li et al., 2015a, b; Cao et al., 2014; Lu et al., 2012). However, as pharmacokinetic study indicated, curcumin (cur), the main active component found in jianghuang metabolites much faster than many other kind of anti-hyperlipidemia medications. Besides, according to chemical and physical properties of curcumin, its solubility in water was not quite satisfied for absorption in body (Li et al., 2015a, b; Gong et al., 2013; Hu et al., 2012). This phenomenon hindered its application in treatment, leading to larger dose of the herb that accumulates toxicity. In order to strengthen the concentration and retention of curcumin in vivo, additive can be used in the guidance of TCM compatibility theory (Li et al., 2015a, b).

Hyperlipemia is one of the commonest threats to human health, which leads to many inconveniences, inefficiencies even fatal consequences. Until recently, numerous researches on its prevention, therapy and mechanism were done by various groups (Chu et al., 2015; Haffner, 1999; La Rovere et al. 1998; Assmann and Schulte, 1992). Contra to chemical medicine that affects one or few targets (Boden et al., 2011; Ginsberg et al., 2010), Traditional Chinese Medicine (TCM) or natural products have comprehensive effects on the body and affecting multiple targets, attracting much attention due to their comprehensive therapy and lower side effect (Liao et al., 2014; Li et al., 2012; Lin et al., 2009; Hong and Ying, 2000). In almost all TCM formulas, couplets are commonly used for strengthening therapeutic effects and/or reducing toxic and side effects (Chen et al., 2014; Li and Xu, 2008).

As previous reports indicated, apart from effects like anticonvulsion, disinfestation and anti-epilepsy, piperine (pip), a compound from *Piper nigrum* L. (The Pharmacopoeia Commission of People's Republic of China, 2015a,b) also has the ability to enhance organisms' absorption of other compounds (Di et al., 2015; Li et al., 2015a, b; Alexander et al., 2014; Johnson et al., 2011; Mittal and Gupta, 2000; Shoba et al., 1998). Li et.al studied the ulcerative colitis therapeutics using curcumin–piperine mixture (Li et al., 2015a, b), which indicated that the co-administration of both compounds improved delivery of the curcumin. Therefore, it is assumed that the problem of solubility and absorption in curcumin can be solved by adding certain amount of piperine and form a formula, which in accordance with TCM theory of "xiangshi" (mutual enhancement)—the addictive acts as "emissary" could strengthen the effectiveness of the main ingredient. However, until recently, few reports about pharmacokinetic characteristic of this curcumin–piperine mixture was seen; nor did the relationship between enhance ability of piperine and its content. In order to establish a scientific guidance for piperine addition and to evaluate its absorption strengthening effect, pharmacokinetic parameters of the curcumin-piperine mixture should be determined and thoroughly studied.

In present study, efforts had been made to achieve fast, sensitive and stable detection of curcumin and piperine in-vivo. Among all options, LC-MS is one of the main technologies with good selectivity, sensitivity and efficiency (Ramesh et al., 2017; Cao et al., 2014; Vijaya et al., 2010; Chen et al., 2012). Attributed to its selective compound monitoring, compounds would no longer require base line separation; in addition, trace level samples can be successfully detected by the instrument due to its much higher sensitivity than HPLC. These advantages are quite practical for pharmacokinetic research, especially for compounds hard to be absorbed and easy to eliminate such as curcumin. Thus, determination of curcumin and piperine in rat plasma will be carried out in LC-MS-MS system. Furthermore, results of our experiment will be used to calculate extent and trend of variations of curcumin's metabolism with and without the presence of piperine, and provide references for scientific TCM compatibility by understanding the mechanism of their interaction.

Material and methods

Chemical and reagents

Curcumin and piperine (98% purity, for method validation) were purchased from Sigma-Aldrich (Darmstadt, Germany); verapamil (for internal standard, 98% purity) was purchased from National Institutes for Food and Drug Control (Beijing, China). Curcumin and piperine for animal experiment (90% purity) were purchased from Zhongsheng beikong Biotech company (Tianjin, China). Reagents for sample pretreatment (methyl tert-butyl ether and ethyl acetate, HPLC grade) and LC-MS determination (acetonitrile and formic acid, HPLC grade) was purchased from Merck (Darmstadt, Germany), deionized water was prepared by Millipore system (France). All other chemicals and reagents was analytical grade.

Animals

SD Rats used for pharmacokinetic study were purchased from Guangdong experimental animal center (Guangzhou, Guangdong), weighing from 230 ± 20 g and sex in half. Before experiment, the rats were raised in SPF laboratory and fed with regular diet. The animal welfare and experimental procedures were strictly in accordance with the guide for the care and use of laboratory animals and the related ethical regulations of the Guangdong Province Engineering Technology Research Institute of T.C.M. 6 of all the 48 rats was continuously fed with regular diet, and given an oral dose of 600 mg/kg of curcumin 30 days later, which used as control group; For building the hyperlipidemia model, other rats were fed with high fat diet for 30 days, and blood was taken from caudal marginal vein to test TC and TG level; then, animals with obvious increase in TC and TG ($p > 0.05$, compared with normal group), were further divided randomly into the following groups (6 rats each, equal in gender): curcumin group, low ratio curcumin-piperine group (c-p, 20:1, 600 mg/kg for curcumin), medium ratio c-p group (10:1) and high ration c-p group (5:1); moreover, all C-P groups were prepared in duplicate for simultaneously giving cur and pip and taking pip 6 h before cur, respectively. Blood of all groups was taken by left common carotid artery intubation at the time point of 0.25, 0.5, 1, 2.0, 4.0, 8.0, 12.0 hours (200 µl each). Finally, the rats were sacrificed, and blood was centrifuged at 8000 rpm to obtain plasma, followed by addition of heparin then stored at -20 °C.

Pretreatment of biological samples

100 µl of the plasma samples obtained in Section "Animals" was transferred into a 1.5 ml centrifuge tube, followed by consecutive addition of 10 µl IS (verapamil, 2 µg/ml, in methanol) and 400 µl methyl tert-butyl ether. Then, the mixture was vortex for 3 min to extract the substances of interest. After extraction, 300 out of all 400 µl of the upper methyl tert-butyl ether was transferred into another clean 1.5 ml centrifuge tube and dried by nitrogenthen redissolved by 100 µl of methanol containing 1% formic acid (v/v).

Determination of curcumin and piperine

Chromatographic separation was carried out by a Thermo Accela pump (Thermo-Fisher Scientific, San Jose, CA, USA) using an Agilent Poroshell SB-C18 (4.6 mm × 150 mm, 2.7 µm) column with a mobile phase of 1% formic acid and acetonitrile 40:60 (v/v) at the