Absorption and Elimination of Dimethoxycurcumin, An Active Analogue of Curcumin

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Abstract. Dimethoxycurcumin (DMC) is an active analogue of curcumin with superior efficacy in various disease models. However, pharmacokinetic research on DMC is limited. The present study was designed to investigate oral absorption and excretion of DMC, as well as making a thorough examination on in vivo metabolism. DMC in rat plasma, bile, urine and feces was quantitated by High performance liquid chromatography (HPLC), and liquid chromatography-mass spectra (LC-MS) detection was performed for metabolites identification. The results showed that the oral bioavailability of DMC was 15 times higher than curcumin. DMC was metabolized in vivo via a similar pathway to curcumin. However, accumulated excretion of DMC was 13.21±1.83% in bile, and the metabolic degree was less extensive than curcumin. Excretion of DMC was less than 1% in urine, and about 65% in feces. All the findings indicated similar in vivo disposition characteristics of curcuminoid.

Introduction

Curcumin is a natural polyphenol extracted from the rhizome of Curcuma longa, and is one of major curcuminoid derivatives with anti-inflammatory, antioxidant, and antimicrobial activities. Since it was identified in early 1900 by Lampe and Milobedzka, curcumin has widely been paid extensive attention due to its broad spectrum of pharmacological activities as a traditional medicine. A series of studies have suggested that curcumin could fight or prevent various types of cancer cells[1]. Despite its multi-faceted pharmacological activities, clinical application of curcumin has been severely hindered due to its limited oral bioavailability, which mainly resulted from low solubility in aqueous solution (2.99×10⁻⁸ M) and metabolic instability. To improve bioavailability, great attention has been paid on the research and development of curcumin analogues, which could overcome the bioavailability drawbacks to some degree, meanwhile retain drug safety of curcumin, increase antitumor activities and other pharmacological effects.

DMC is a kind of lipophilic analogue of curcumin with all phenolic hydroxyl groups methylated, it could be easily obtained by synthetic or semi-synthetic methods. Plenty of studies have shown that DMC possesses many pharmacological activities, even more
effective in fighting numerous cancer cells, and the antitumor mechanism is similar to its parent compound curcumin[2]. Even at a very low concentration, DMC could induce epigenetic changes in leukemia cells, and the effect was ten-fold higher than curcumin[3].

In view of the clinical limitation of curcumin, the absorption of DMC was unknown, besides, in vivo metabolism and excretion had not been reported to the best of our knowledge. Tamvakopoulos[4] has analyzed the in vitro metabolic pathway and plasma metabolic stability of DMC, which demonstrated that metabolism of DMC was less extensive in liver microsomal systems, and the plasma metabolic stability was more stable than curcumin. However, deficient factor of consistency induces external metabolism could not completely reflect internal condition, since the latter is more complex and variable, and the quantity of pro-type drug was either indistinct. So the results of external used to predict DMC metabolism would still need internal metabolism experiment for further confirmation.

Aim of the present study was to further analyze oral absorption, in vivo metabolism and excretion of DMC, determining its oral bioavailability, in vivo metabolic reaction and degree, and main excretion pathway in comparison with curcumin, to enriched basic researches of DMC and to substantiate the better medical advantage of DMC in pharmacokinetic aspect.

Materials and Methods

Chemicals

DMC, bis-demethoxycurcumin used as an internal standard (IS), were prepared in-laboratory and tested for purity, which was > 98.0%. Lyophilized DMC polymeric micelles (DMC-PMs, 20 mg/vial) for injection were prepared by our laboratory, which have been well characterized in our previous research with high drug loading and stability. Acetonitrile, methanol and formic acid were of HPLC grade and purchased from Sigma-Aldrich Co., LLC. (St. Louis, MO, US). Sodium carboxymethyl cellulose (CMC-Na) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals were reagent grade and were used as received.

Animals

Male Sprague Dawley (SD) rats weighing 200-250 g were purchased from the Laboratory Animal Services Center of Luye Pharma Group Ltd. (Yantai, China). All animals used were exposed to a 12-hour light/dark cycle in a specific pathogen-free environment and received food and water ad libitum throughout the studies.

Samples Collection and Preparation

All rats were randomly devided into four groups (n=6). Group I were administered a single intravenous (i.v.) dose (10mg DMC/kg of body weight) of DMC-PM solution, Group II were orally administered (p.o.) 100 mg/kg of body weight of DMC in CMC-Na suspension. At various time points after dosing, serial plasma were collected. Group III were secured and implanted with PE-10 cannulas into bile ducts under anesthesia by ether. Group IV feeded in metabolic cages. Blank bile, urine and feces were collected, respectively. Then were administered a single i.v. dose (20mg DMC/kg of body weight) of DMC-PM. After that, bile, urine and feces samples were collected. All biological samples were stored at -20℃ for further analysis.
Sample Preparation

Briefly, 100 μL of plasma, bile, urine and fecal specimen homogenate samples was mixed with 10 μL of IS, 200 μL of hydrochloric acid and 3 mL of ethyl acetate. The mixtures were vortexed, ultrasonic extracted, and were then centrifuged. After that, the organic layer was transferred and evaporated to dryness. Extraction residue was reconstituted and injected into HPLC system.

Quantification of DMC by HPLC

DMC concentrations were determined by HPLC. Samples were injected into Agilent 1100 HPLC system. Measurements were carried out on Luna column; Mobile phase was acetonitrile-0.1% formic acid water with a flow rate of 1 mL·min⁻¹; Detection wavelength was 420 nm. Detection methods of DMC in all biological samples were fully validated, and the results indicated that all assays could be applied to the quantification of DMC in plasma, bile, urine and feces.

Metabolites Identification by LC-MS

DMC metabolites were conducted on LC-MS system. HPLC operating conditions for the same. MS acquisition was performed on fisher triple stage quadrupole MS in scan mode of positive ions and operating conditions were optimized.

Statistical Analysis

The plasma concentration-time data were analyzed with a non-compartmental model by DAS 2.1 software to obtain pharmacokinetic parameters. The absolute oral bioavailability (F) was generally measured.

Results and Discussion

Pharmacokinetic Study

As plasma concentration versus time profiles of DMC with a single i.v. and p.o. dose administration to each group illustrated in Fig.1, the concentration-time curve of p.o. administration presented significant absorption stage. AUC values were 46.27±1.53 mg·h·L⁻¹ and 69.58±3.18 mg·h·L⁻¹ for i.v. and p.o. administration, respectively. Half-life (t₁/₂) of p.o. was 3.85±1.07 h, which was longer than i.v. administration. After administration 1 h, maximum concentration (C max) of p.o. arrived at 35.21±1.01 mg·L⁻¹, C max of i.v. administration were 42.77±1.22 mg·L⁻¹ in the beginning. According to calculating, F value of DMC was 15.04%. Yang[5] has reported oral bioavailability of curcumin was about 1%, 15 times higher bioavailability than curcumin might indicated better medical potential of DMC.
Identification of DMC Metabolites in vivo

DMC metabolites were extracted and analyzed by LC-MS. Full scan mass spectra of rat bile after administration of DMC was compared with blank samples to find the possible metabolites. The results showed that except for parent drug, there were about seven new peaks appeared, which might be attributed to DMC metabolites. The possible structures were shown in Table 1.

Table 1. HPLC-MS data and proposed compounds of DMC metabolites in rat bile.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>T&lt;sub&gt;r&lt;/sub&gt;[min]</th>
<th>m/z</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>15.34</td>
<td>406.99 [M+K&lt;sup&gt;+&lt;/sup&gt;]</td>
<td>Bisdemethoxy-DMC (Curcumin)</td>
</tr>
<tr>
<td>M2</td>
<td>9.04</td>
<td>423.15 [M+K&lt;sup&gt;+&lt;/sup&gt;]</td>
<td>Dihydro-demethoxy-DMC</td>
</tr>
<tr>
<td>M3</td>
<td>7.40</td>
<td>493.08 [M+K&lt;sup&gt;+&lt;/sup&gt;]</td>
<td>Hexahydro-bisdemethoxy-DMC sulfate</td>
</tr>
<tr>
<td>M4</td>
<td>6.26</td>
<td>401.02 [M+H&lt;sup&gt;+&lt;/sup&gt;]</td>
<td>Tetrahydro-DMC</td>
</tr>
<tr>
<td>M5</td>
<td>5.91</td>
<td>371.02 [M+H&lt;sup&gt;+&lt;/sup&gt;]</td>
<td>Dihydro-bisdemethoxy-DMC</td>
</tr>
<tr>
<td>M6</td>
<td>3.96</td>
<td>466.15 [M+H&lt;sup&gt;+&lt;/sup&gt;]</td>
<td>Tetrahydro-demethoxy-DMC sulfate</td>
</tr>
<tr>
<td>M7</td>
<td>2.47</td>
<td>603.05 [M+K&lt;sup&gt;+&lt;/sup&gt;]</td>
<td>Hexahydro-demethoxy-DMC glucuronide</td>
</tr>
</tbody>
</table>

Based on the above analysis, proposed metabolic pathway of DMC in rat was presented in Fig.2. *In vivo*, DMC degrade to curcumin rapidly, and the degree of subsequent reaction was also different to external environment. Likewise, other O-demethylation reactions of DMC *in vitro* were also deficient. For example, after glucuronidation, the reduction reaction was almost stopped *in vitro*, but hydrogenation reaction was still continued *in vivo*. In addition, direct reduction reaction of DMC was also slow, and dihydro-DMC was detected before it transformed into tetrahydro-DMC, but only tetrahydro-DMC was detected *in vivo*. The internal reaction might be faster, and metabolic degree was deeper, so internal reaction might be more complex due to variable factors and environment *in vivo*.
Excretion of DMC in Bile, Urine and Feces

Rat bile, urine and feces excretion of DMC pro-type drug after i.v. administration DMC were illustrated in Fig. 3. Results shown that accumulative bile excretion rate of DMC pro-type drug was 13.21%±1.83%. After i.v. administration DMC into rats, most DMC were excreted by pro-type in bile samples. About 55% pro-type drug were accumulated in 0-0.5 h, after administration 24 h, there were still 0.05% parent drug excreted in rat bile. Excretion of DMC in urine was only 0.76%±0.13%, while 64.63%±7.48% in feces within 72 h, and excretion rate of DMC was total about 66% both in urine and feces. After i.v. administration DMC 72 h, DMC still could be detected in feces, which further indicated high in vivo metabolic stability and absorption of DMC.

It has been reported that curcumin was quickly metabolized into tetrahydrocurcumin and other low activity products, and the pro-type drug could not be detected in bile, and this might be the reason of low bioavailability of curcumin. Tamvakopoulos[4] has reported that plasma stability of DMC was about 3 times higher than curcumin, but the metabolic degree of DMC has not provided. As curcumin was rapid metabolized, the lower in vivo metabolic degree of DMC further expounded it was stable enough to exert therapeutic function. Higher metabolic stability endow DMC more effective pharmacological activities and higher bioavailability than curcumin. Due to low solubility, the excretion rate of DMC in urine was limited. Anand[6] shown that >70% curcumin were excreted by feces, >10% were excreted by bile, and the quantification of curcumin in urine was rarely. Therefore, excretion pathway of DMC was similar to curcumin in vivo, and the curcuminoind compounds might have the same excretion pathways.
Conclusions

In view of the disadvantages of curcumin, DMC as a more effective analogue was studied in the present research. We analyzed the oral bioavailability of DMC, and the 15 times higher bioavailability proved better clinical medicine application. Metabolism and excretion of DMC \textit{in vivo} were firstly analyzed in the research and results shown that internal metabolism of DMC might be more complex, the metabolic degree of pro-type further explained the stability of DMC. Metabolism and main excretion pathway of DMC and curcumin were almost similar \textit{in vivo}.

In addition to excellent pharmacological activity, higher oral bioavailability and metabolic stability implied higher medicinal properties in pharmacokinetics aspect, and the advantages could make up the clinical application limits of curcumin in some degree, which means greater medicinal possibility and medicinal development value of DMC.

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