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Pharmacokinetics Analysis of Copen, a Novel Antitumor Semi Synthetic Derivative of Osthole, in Rats after Intragastric and Intravenous Administration

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Abstract

Copen is one of the major semi synthetic derivatives of osthole with obvious antitumor activity. The absolute bioavailability and gender-related pharmacokinetic properties of copen in rats were determined in this study. Sprague-Dawley rats were intragastrically and intravenously administrated of different doses of copen, respectively. The concentrations of copen in rat plasma were determined by a LC-MS/MS method. Pharmacokinetic parameters were estimated using a drug and statistics (DAS) software. Statistical analysis was performed using independent two-sample t-test with p-values less than 0.05 as the level of significance. The results indicated that maximum plasma concentrations (C_{max}) for copen were achieved at 9.17-14.17 min post-intragastric dosing; the elimination half-life ($t_1/2_z$) of copen after intragastric dosing was 196.55-302.16 min. After intragastric administration of copen, the spearman's rank correlation coefficient (rs) of C_{max}-Dose was 0.49810 (p=0.0023), and the rs of AUC_{0-t}-Dose was 0.74634 (p<0.0001). Significant differences (p<0.05) of AUC_{0-t}, $AUC_{0-\infty}$, CLz/F and C_{max} were present in female and male groups after intragastric doses. Absolute bioavailability of copen was assessed to be ranged from 2.21-10.67% for different doses in rats. The pharmacokinetic properties of copen in rat were characterized as rapid oral absorption, slow clearance, and significant gender differences.

Keywords: Pharmacokinetics; Absolute bioavailability; Gender difference; Copen

Introduction

Nowadays, the active compounds from Traditional Chinese Medicine (TCM) have attracted more and more attention. Osthole (Figure 1) is a major bioactive ingredient isolated from *Cnidium monnieri* (L.) cusson [1,2], which has been used for treatment of pain in female genitalia, impotence, and suppurative dermatitis (as an antipruritogenic agent). Osthole exhibits various pharmacological activities, including *in vitro/in vivo* antitumor effects [3-8], alleviation of hyperglycemia and hypolipidemia [9,10], and has been proposed the possibility of its development as a promising lead compound for drug discovery [11,12]. However, the clinical utility of this phytochemical is limited due to its low bioavailability *in vivo* [13-16], and some structural modifications are required for sufficient bioavailability upon oral administration. Copen (Figure 1) is a semi synthetic derivative of osthole obtained by structural modification of the compound, which showed a relatively obvious proliferation inhibition effect on 95-D, Bel-7402, MDA-MB-231, PC-3 and HL-60 tumor cells with the IC50 value less than 15 μM (determined in our lab), and was considered as a new antitumor drug candidate [17,18]. The further investigations of copen were funded by National Major Scientific and Technological Special Project for "Significant New Drugs Development" in China, Project No. 2011ZX09302-003-03.

Previous research in our team revealed that copen demonstrated good antitumor activity on 95-D, Bel-7402, MDA-MB-231, PC-3, and HL-60 tumor cells. In order to elucidate the mechanism of its pharmacological activity and toxicity, it's very necessary to investigate the pharmacokinetics of copen *in vivo* since limited data was available. Our previous study reported a quantification method for copen in rat plasma and its simple application [19], which was not enough for the illustration of the systematic pharmacokinetic behavior of copen in rats. Copen is presently undergoing preclinical study; therefore, it is necessary to evaluate the pharmacokinetics and oral bioavailability of copen for further development.

In the present study, the pharmacokinetics of copen after intragastrical and intravenous administration were systematically investigated using a LC-MS/MS method. Key pharmacokinetic issues, such as oral bioavailability and gender difference, would be well addressed in support of the further structural modification of osthole and copen.

Materials and Methods

Chemicals and reagents

Copen (purity >99.5%, Lot No. 20131210) was provided by Guangdong Zonk Drug R&D Limited, and bicalutamide (purity >99.3%) was kindly provided by Zhejiang Institute for Food and Drug Control (Hangzhou, PR China) (Figure 1). Ultrapure water (>18 mΩ) was obtained from a Milli-Q water purification system (Millipore, MA, USA). HPLC grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). All other chemicals used were of analytical grade.

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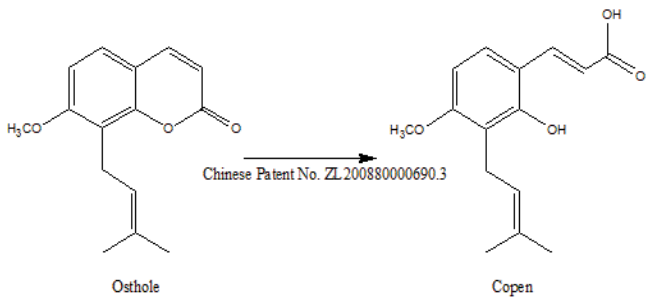

Figure 1: Chemical structures of copen and osthole.

Animals

Sprague-Dawley adult healthy rats (weighing 211 ± 13 g) both male and female were purchased from the Laboratory Animal Center of Zhejiang Academy of Medical Sciences (Hangzhou, China). Animals were raised under controlled conditions in the Institute for Experimental Animals, First Affiliated Hospital, Zhejiang University (temperature: 25 ± 2°C, 12 h light-dark cycle), for at least one week before experiment. All experiments performed in rats were in accordance with the P.R. China legislation on the use and care of laboratory animals and approved by Experimental Animal Ethical Committee, First Affiliated Hospital, Zhejiang University.

Study design

Seventy-six Sprague-Dawley rats, both male and female (211 ± 13 g), were fed with standard laboratory food and water, and kept in an environmentally-controlled breeding room for six days before experimentation. Rats were fasted for 12 h and allowed free access to water prior to the experiments.

The rats were randomly divided into six groups and twelve rats (50% male and 50% female) in each group. Three groups were given copen (62.5 mg/mL, dissolved in normal saline) intragastricly at doses of 25, 50 and 100 mg/kg, while the other three groups were given intravenously (through tail vein injection) at the same doses, respectively. The doses were confirmed according to the results from our previous *in vivo* tumor inhibition effect of copen in mice. Disposable sterilized syringes were used for intravenous administration and medical cotton ball was pressured on the wound until bloodless after injection. Blood samples (about 300 μL) were collected in heparinized 1.5 mL polythene tubes from the tail vein before and at 0, 5, 10, 15, 30, 60, 90, 120, 240, 360, 600 and 1440 min after intragastrical and intravenous administration. All blood samples were immediately centrifuged to separate plasma at 3000 rpm at 4°C for 10 min. The plasma was transferred into clean tubes and stored at -80°C until analysis.

Copen analyses

Plasma concentrations of copen were determined by using an LC-MS/MS method (Shimadzu LC-20AD system, Shimadzu, Tokyo, Japan) validated in our laboratory [19]. Copen and internal standard (bicalutamide) were separated on an Agilent ZorbaxSB-C18 column (4.6 × 100 mm, 1.8 μm, Agilent Technologies, CA, USA), using an isocratic mobile phase consisting of methanol -5 mM ammonium formate water with 0.1% formic acid (80:20, v/v). Column temperature was maintained at 40°C. The detection was performed on a triple-quadrupole tandem mass spectrometer (API 4000 triple-quadrupole mass spectrometer; AB Sciex, Ontario, Canada) by the multiple reactions monitoring mode via negative eletrospray ionization. The parent and daughter ions transitions were monitored at m/z 261.2→217.1 for copen and at m/z 429.0→255.0 for bicalutamide (IS). Two linear calibration curves were obtained in the concentration range of 0.052 ~ 20.6 μg/mL (using a 1/x2 weighted least squares linear regression model) and 10.1 ~ 504.8 μg/mL (using a 1/x weighted least squares quadratic regression model). The intraday and interday precision values (R.S.D%) were within 15%.

Dilution integrity was carried out in this study. The blank plasma samples spiked with 1010 μg/mL of copen (exceeding the upper limit of the calibration curve) were diluted by five-fold and subjected to LC-MS/MS analysis. The experiments were conducted in six replicates. The dilution integrity was deemed acceptable when the precision and accuracy were within ± 15%.

Statistical analyses

All experimental data and pharmacokinetic parameters are expressed as the means ± Standard Deviation (SD). Peak plasma concentrations (C_{max}) and the time to reach peak plasma concentrations (T_{max}) were obtained directly from the observed concentration versus time data. Other pharmacokinetic parameters were estimated through the drug and statistics (DAS) software, version 2.1.1 (Shanghai, China). Bioavailability of different gender was calculated individually. Statistical analysis of the pharmacokinetic parameters was performed with SPSS 16.0 software using independent two-sample t-test, p-values less than 0.05 were considered statistically significant.

Results

Dilution integrity

The concentration of copen in some plasma samples exceeded the upper limit of detection, therefore, these samples were diluted by five-fold in the same biological matrices prior to LC-MS/MS analysis. We determined the accuracy and precision of copen detection using diluted biological samples. The accuracy was between 96.4% and 105.6%, and the precision was within 3.29% for samples diluted in plasma matrices. These results indicated that the concentration of copen in plasma could be determined with acceptable precision and accuracy using diluted samples.

Sample analysis and quality control (QC)

New linear calibration curves were constructed during every day's analyzing, and QC samples at six concentration levels (0.052, 1.65, 16.5 μg/mL for the low concentration calibration and 4.04, 40.4, 404 μg/mL for the high concentration calibration, n=2) were analyzed at the same time. The results of QC samples all showed good precision and accuracy within the 15% acceptable range, which indicated the concentrations of copen determined in this study were reliable.

Pharmacokinetic analysis

Pharmacokinetics of copen in rats after intragastric administration: The mean plasma concentration-time curves of copen in rats after intragastric administration at doses of 25, 50 and 100 mg/kg was shown in Figure 2. The main pharmacokinetic parameters of intragastric administrations were summarized in Table 1.

Pharmacokinetics of copen in rats after intravenous administration: After intravenous administration of copen at doses of 25, 50 and 100 mg/kg to rats, the mean plasma concentration-time profiles of copen are shown in Figure 2. The main pharmacokinetic parameters after intravenous administration are listed in Table 2.

Influence of gender on the pharmacokinetics of copen: The mean plasma concentration-time curves and pharmacokinetic parameters of copen in male and female rats after intragastric doses of copen are shown in Figure 3 and Table 3, respectively.

Bioavailability and safety

The absolute bioavailability (F%) of copen was calculated by comparing the corresponding values of $AUC_{0-\infty}$ from the intragastric (i.g) and intravenous (i.v) administration groups (25, 50 and 100 mg/kg): $(F\%=(AUC_{i.g})/(AUC_{i.v}) \times 100\%)$, and the results of the absolute bioavailability are shown in Table 4. The results showed that intragastric administration of copen leads to an absolute bioavailability of 2.21 ~ 10.67%. The absolute bioavailability in females was higher than that in males, especially for the high dose (100 mg/kg). This observation suggests that copen exhibited a better absorption property in females than in males, which was in agreement with the high AUC and C_{max} estimates.

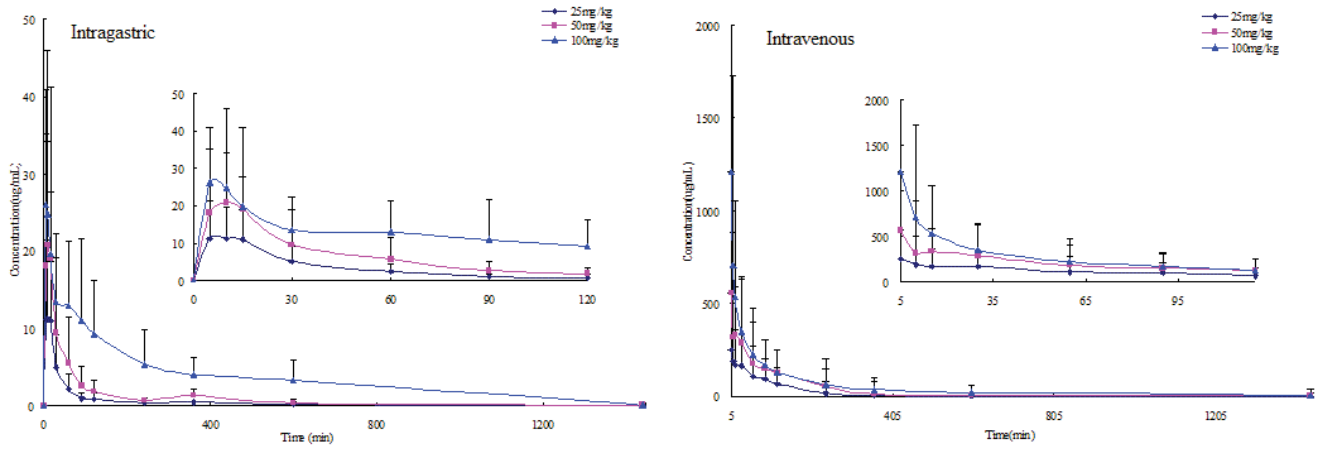

Figure 2: The mean plasma concentration-time curves of copen in rats after intragastric and intravenous doses of copen (25, 50 and 100 mg/kg; n=12).

Parameters \ Doses	25 mg/kg	50 mg/kg	100 mg/kg
AUC_{0-t} (mg/L min)	729.59 ± 437.80	1468.60 ± 920.76	5167.31 ± 2842.48
$AUC_{0-\infty}$ (mg/L min)	1018.02 ± 421.57	1546.33 ± 957.81	5708.30 ± 10770.80
MRT_{0-t} (min)	199.84 ± 108.14	215.88 ± 135.95	292.55 ± 80.53
$MRT_{0-\infty}$ (min)	305.00 ± 208.98	264.41 ± 151.59	409.51 ± 368.18
$t_{1/2z}$ (min)	302.16 ± 169.90	243.80 ± 112.71	196.55 ± 66.44
T_{max} (min)	10.00 ± 3.69	9.17 ± 3.59	14.17 ± 24.01
CLz/F (L/min/kg)	0.046 ± 0.027	0.049 ± 0.031	0.025 ± 0.016
C_{max} (mg/L)	13.39 ± 10.17	21.96 ± 25.84	28.35 ± 10.24

Table 1: Pharmacokinetic parameters of copen after intragastric administration of 25, 50 and 100 mg/kg (n=12, mean ± SD).

Parameters \ Doses	25 mg/kg	50 mg/kg	100 mg/kg
AUC_{0-t} (mg/L min)	24545.80 ± 21102.82	54342.71 ± 76451.41	68653.62 ± 37177.46
$AUC_{0-\infty}$ (mg/L min)	24813.01 ± 21056.08	57579.07 ± 81167.16	83932.17 ± 60623.48
MRT_{0-t} (min)	82.90 ± 54.72	111.83 ± 57.71	122.012 ± 62.79
$MRT_{0-\infty}$ (min)	199.53 ± 348.08	138.60 ± 87.58	235.61 ± 375.14
$t_{1/2z}$ (min)	236.49 ± 101.77	258.07 ± 110.36	229.05 ± 110.11
T_{max} (min)	5.00 ± 0.0	5.00 ± 0.0	5.00 ± 0.0
CLz (L/min/kg)	0.007 ± 0.009	0.003 ± 0.003	0.003 ± 0.005
C_{max} (mg/L)	338.34 ± 290.40	553.68 ± 539.87	1451.58 ± 1108.34

Table 2: Pharmacokinetic parameters of copen after intravenous administration of 25, 50 and 100 mg/kg (n=12, mean ± SD).

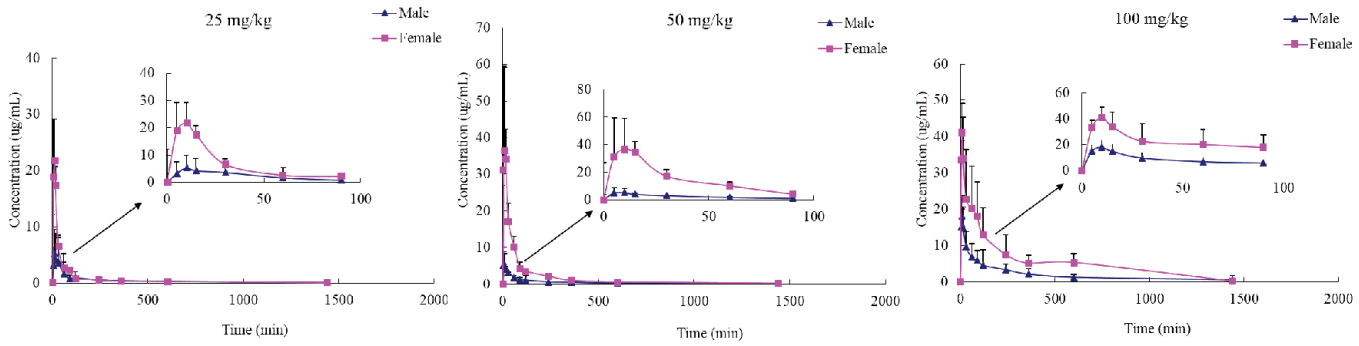

Figure 3: The mean plasma concentration-time curves of copen in male and female rats after intragastric doses of copen (25, 50 and 100 mg/kg; n=6).

Gender	Doses	Parameters					
		AUC_{0-t} (mg/L min)	$AUC_{0-\infty}$ (mg/L min)	$t_{1/2z}$ (min)	T_{max} (min)	CLz/F (L/min/kg)	C_{max} (mg/L)
Male	25 mg/kg	570.16 ± 532.90	517.17 ± 504.41	364.20 ± 200.12	10.83 ± 3.76	0.048 ± 0.024	5.79 ± 4.67
	50 mg/kg	726.83 ± 337.32	770.38 ± 383.57	276.91 ± 133.51	8.33 ± 4.08	0.074 ± 0.022	5.41 ± 3.74
	100 mg/kg	2917.47 ± 1082.43	2939.62 ± 1065.02	173.58 ± 70.11	6.67 ± 2.58	0.037 ± 0.011	19.40 ± 4.82
Female	25 mg/kg	889.01 ± 276.92	1518.86 ± 286.24	240.11 ± 119.23	9.17 ± 3.76	0.032 ± 0.017	20.98 ± 8.20*
	50 mg/kg	2210.37 ± 656.25*	2322.29 ± 652.76*	210.70 ± 86.57	10.00 ± 3.16	0.023 ± 0.007*	38.52 ± 28.23*
	100 mg/kg	7417.15 ± 2110.77*	8476.98 ± 3691.84*	219.52 ± 59.41	21.67 ± 33.57	0.012 ± 0.008*	37.30 ± 3.88*

*Indicates statistical significance (p<0.05).

Table 3: Pharmacokinetic parameters of copen in male and female rats after intragastric doses of copen (25, 50 and 100 mg/kg; n=6).

Rats sex	F % (25 mg/kg)	F % (50 mg/kg)	F % (100 mg/kg)
Male	3.51	2.21	3.32
Female	4.35	2.89	10.67

Table 4: Absolute bioavailability of copen in female and male rats (n=6).

Throughout the study, no died rat was found. However, after intragastrical and intravenous administration, all the rats' tails appeared different degrees of necrosis, especially the site of injection, which indicated that copen have obvious skin toxicity.

Discussion

After intragastric administration of copen, the absorption of copen from the rat gastrointestinal tract was discovered rapidly. Copen was detected in the plasma from the first blood sampling time (5 min) and rapidly reached T_{max} (9.2-14.2 min). It is worthy of noting that the spearman's rank correlation coefficient (rs) of C_{max}-Dose was 0.49810 (p=0.0023), and the rs of AUC_{0-t}-Dose was 0.74634 (p<0.0001), indicating a positive correlation between C_{max} or AUC_{0-t} and intragastric doses.

After intravenous administration of copen, the elimination half-lives ($t_1/2_z$) were assessed to be 236.49 ± 101.77, 258.07 ± 110.36 and 229.05 ± 110.11 min at doses of 25, 50 and 100 mg/kg to rats, respectively. It indicated that copen has a long dwell time in rat with a long elimination half-life (about 4 h). Total clearance (CLz) of copen was estimated to be 0.003 ~ 0.007 L/min/Kg among the doses tested (Table 2). The spearman's rank correlation coefficient (rs) of C_{max}-Dose was 0.64171 (p<0.0001), and the rs of AUC_{0-t}-Dose was 0.64337 (p<0.0001), which also showed strong positive correlation between C_{max} or AUC_{0-t} and intravenous doses.

As big SD were observed for most of the parameters in Table 1 and Table 2, the differences of gender on copen's absorption or distribution were analyzed. The results showed that significant differences (p<0.05) of AUC_{0-t}, $AUC_{0-\infty}$, CLz/F and C_{max} were present in female and male groups after intragastric doses (50 and 100 mg/kg). The results suggested that copen possessed more favorable absorption properties in females than in males, as the values of AUC and C_{max} were more than 2-fold higher in females than in males. The mechanism of this phenomenon needs further studies to be clarified.

Compared with the $AUC_{0-\infty}$ in rats after intravenous and intragastric administration of copen at doses of 25,50 and 100 mg/kg, the absolute bioavailability were 4.10%, 2.69%, 6.80% (n=12), respectively, which were lower than that of osthole (F%=15.65%), indicating the lactone hydrolysis could not help osthole improving its bioavailability. Further structure modification must be carried out based on copen or osthole, so as to improve their bioavailability and reduce their toxicity.

Conclusion

This is the first study on the investigation of absolute bioavailability, pharmacokinetics, and gender difference of copen. Positive correlation between C_{max} or AUC_{0-t} and doses were noted. The long half time (229.05 ~ 258.07 min) indicates that the elimination of copen is not rapid. Significant gender differences were observed in pharmacokinetic behaviors of copen in rats after intragastric administration, as females performed much better absorption properties than males. Furthermore, the absolute bioavailability of copen in male and female rats was estimated as 2.21 ~ 10.67%. The presence of obvious gender difference

in absorption properties and skin toxicity may have an impact on the further structure modification of osthole and copen, and should be considered into the development of therapeutic regimens.

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