Journal of Food and Drug Analysis, Vol. 13, No. 1, 2005, Pages 38-42 藥物食品分析 第十三卷 第一期

Pharmacokinetics of A New Antitumor Agent, 1-[3-(Furo[3,2-C]quinolin-4-ylamino)phenyl]-ethanoe -*O*-methyl-oxime, in Rat Using A High Performance Liquid Chromatography Method

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(Received: September 16, 2004; Accepted: December 21, 2004)

ABSTRACT

1-[3-(Furo[3,2-C]quinolin-4-ylamino)phenyl]ethanoe-*O*-methyl-oxime (CCK3) is an antitumor agent, particularly active against the growth of the renal cancer cell, UO-31, and two melanoma cancer cells, UACC-257 and UACC-62, as indicated in the NCI's full panel of 60 human cancer cell lines cytotoxicity evaluation. From the structure-activity relationships between amsacrine and CCK3, CCK3 was expected to have longer half-life than amsacrine in plasma. Therefore, a reversed-phase high performance liquid chromatography method was developed and validated for the determination of pharmacokinetics of CCK3 in rats. The plasma samples were spiked with the internal standard 2-naphthol and extracted using dichloromethane. A C18 column (55 × 4 mm) was used for the separation of analytes with a mobile phase consisted of 30% acetonitrile, 5% tetrahydrofuran and 65% pH 3.0 of McIlvaine buffer at a flow rate of 1.0 mL/min. CCK3 was detected and utilized by electrochemical detector at 1.0 voltage and 10 nA. Intra- and inter-day precision and accuracy were acceptable down to the limit of quantitation of 10 ng/mL. The lower limit of detection was 5 ng/mL. As for the *in vivo* study, the pharmacokinetic parameters of CCK3 in rats after intravenous administration of 3.97 and 7.94 mg/kg were determined. The apparent volume of distribution, half-life and clearance showed no significant difference between these two dosages. The area under the plasma concentration time curve increased proportionally with increase in dose. The half-life of CCK3 was prolonged 3 folds, compared to amsacrine. Therefore, CCK3 might have the potential to be tested clinically.

Key words: 1-[3-(furo[3,2-C]quinolin-4-ylamino)phenyl]-ethanoe-*O*-methyl-oxime, amsacrine, antitumor, pharmacokinetics, half-life

INTRODUCTION

Amsacrine is a substituted 9-aminoacridine derivative which has clinical activity against acute lymphocytic and nonlymphocytic leukemias[(1-5)]. However, the half-life of amsacrine in plasma is relatively short about 0.5 hr, resulting in inconvenient clinical use[(6)]. Therefore, a series of structure-activity relationships (SAR) studies had been conducted to obtain a drug with potent antitumor activity and longer half-life in human plasma[(7-11)]. The major route of breakdown for amsacrine and its analogs *in vivo* is a nonenzymatically mediated attack of thiol at C[(9)], eventually resulting in loss of the side chain and the formation of inactive products[(12-14)]. The furo[2,3-b]quinoline possessing a higher electron density than that of acridine was used to replace acridine, and obtained a 4-anilinofuro[2,3-b]quinoline derivative with a longer half-life[(11,15)]. In the structure- activity relationships studies, a series of 4-anilino furo[2,3-b]quinoline derivatives with expected longer half-life in plasma have been synthesized[(16)]. Among these derivatives, 1-[3-(furo[3,2-c]quinolin-4-ylamino)phenyl]-ethanoe-*O*-methyl-oxime (CCK3) was found to be especially active against the growth of one renal cancer cell, UO-31 (GI_{50} < 0.01 μM), and two melanoma cancer cells, UACC-257 (GI_{50} = 0.04 μM) and UACC-62 (GI_{50} < 0.01 μM), in the NCI's full panel of 60 human cancer cell lines cytotoxicity evaluation[(16)], indicating that CCK3 might have the potential to be tested clinically. To show that CCK3 may have a longer half-life, a sensitive and robust method has been developed to study the pharmacokinetics of CCK3 following intravenous administration in rats.

MATERIALS AND METHODS

I. *Materials*

1-[3-(Furo[3,2-c]quinolin-4-ylamino)phenyl]-ethanoe-*O*-methyl-oxime (CCK3) was synthesized in the laboratory of School of Medicinal and Applied Chemistry (Kaohsiung Medical University, Taiwan). 2-Naphthol and tetraglycol were purchased from Sigma (St. Louis, MO, USA).

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Journal of Food and Drug Analysis, Vol. 13, No. 1, 2005

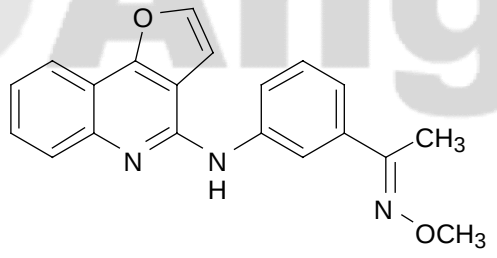

Figure 1. Structure of 1-[3-(furo[3,2-C]quinolin-4-ylamino)phenyl]-ethanoe-*O*-methyl oxime (CCK3.)

Methanol and acetonitrile were obtained from Tedia Co. (USA). 1-Pentanesulphoric acid and Dichloromethane were purchased from Merck (Germany). *N*,*N*-dimethylacetamide was obtained from Mallinckrodt (USA). All other chemicals and solvents were of analytical reagent grade.

II. *Apparatus and Chromatographic Conditions*

An HPLC equipped with a Hitachi model L-7100 pump, a BAS LC-4C electrochemical detector, a Jasco 855-AS autosampler and a Merck Lichrocart® C18 column (55 × 4 mm i.d., 3µ) was used. The mobile phase was a mixture of 30% acetonitrile, 5% tetrahydrofuran and 65% of pH 3.0 McIlvaine buffer (v/v/v), at the flow rate of 1.0 mL/min. The effluent from the column was monitored by an electrochemical detector at +1.0 voltage and 10 nA. BAS MF 1000 dual glassy-carbon working electrode and DAS RE6 Ag/AL reference electrode were used.

III. *Drug Standards*

Standard solutions of CCK3 were prepared in methanol at final concentrations ranging from 100 to 30,000 ng/mL. Plasma standard were prepared using drug-free rat plasma spiked standard solution to obtain the appropriate final concentrations. The internal standard (IS, 2-Naphthol, 1 µg/mL) was prepared in methanol.

IV. *Preparation of Plasma Samples*

IS solution (0.03 mL) and 4 mL of dichloromethane were added to aliquots of 0.2 mL of blank, spiked or plasma samples. The mixture was horizontally shaken for 50 min and centrifuged at 3,000 rpm for 10 min. The organic layer was transferred to another tube and evaporated to dryness by a vacuum pump. The dry residue was reconstituted in 0.2 mL of methanol and 0.02 mL of the clear supernatant that was injected into the HPLC system.

The recovery of CCK3 from plasma was determined by comparing the peak area obtained from spiked CCK3 samples with that from standards in methanol solution.

For method validation, standards of rat plasma were prepared and analyzed. Linearity, intra- and inter-assay precision and accuracy were determined from these data. The limit of detection (LOD) of CCK3 was determined.

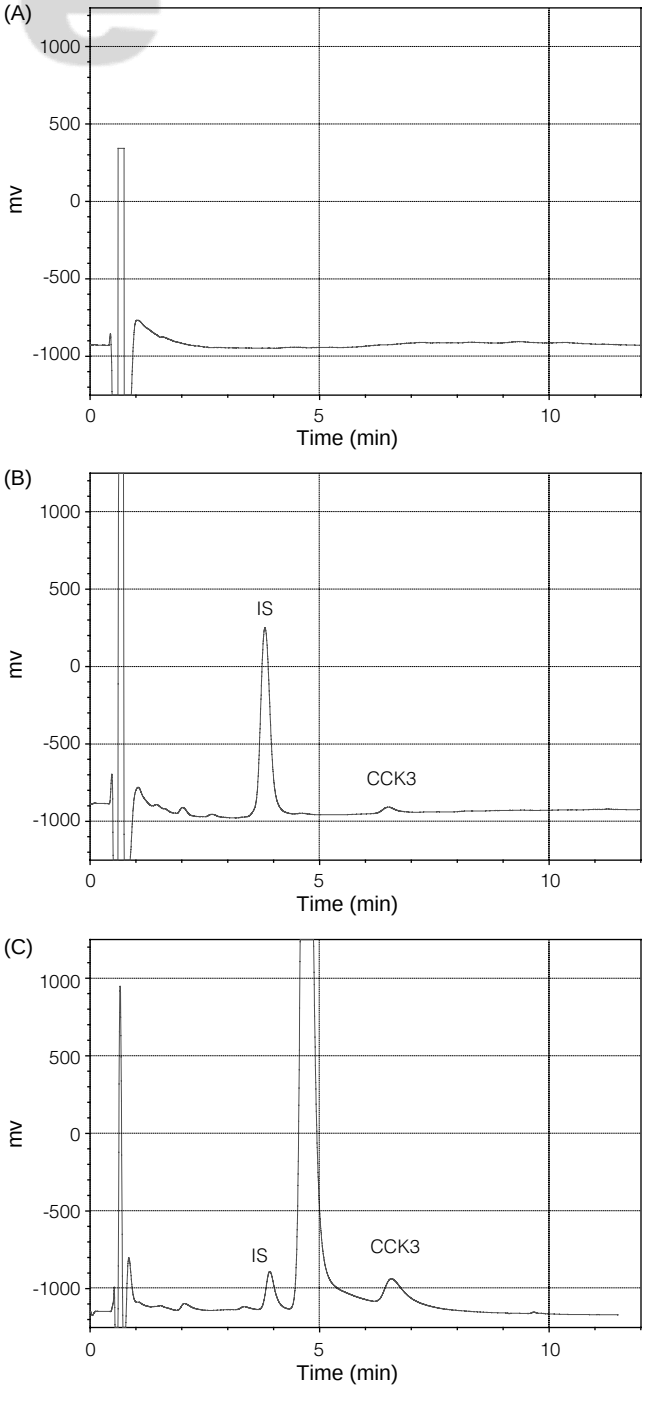

Figure 2. HPLC chromatograms of CCK3 in rat plasma. (A) blank plasma (B) plasma spiked with CCK3 (10 ng/mL) and internal standard (IS) (1 µg/mL) (C) rat plasma extracted for 5 min after IV administration of 3.97 mg/kg of CCK3.

LOD is the smallest concentration where signals can be distinguished from the noise level, with a typically acceptable signal-to-noise of 3:1.

V. *Determination of CCK3 Pharmacokinetics*

Wistar rats weighing 190-220 g were used in the study (Laboratory Animal Center of National Science Council).

Rats were anesthetized by intraperitoneal injection of 25% urethane (3 mL/Kg). CCK3 was dissolved in a aqueous solution containing 10% *N,N*-dimethylacetamide, 30% polyethylene glycol and 30% tetraglycol at final concentrations of 1.6 and 3.2 mg/mL. CCK3 aqueous solution (0.5 mL) was administered *via* the tail vein at dose of 3.97 and 7.94 mg/kg. Blood samples (0.45 mL) were collected from the jugular vein at fixed time intervals before and after CCK3 administration. The blood samples were then centrifuged at 12,000 rpm for 10 min, and then plasma was immediately separated and stored at -20˚C until analysis.

VI. *Analysis of Pharmacokinetic Data*

Concentration-time profiles were analyzed using the PCNONLIN computer program (V4.0, SCI software, USA). The following pharmacokinetic parameters were assessed: the area under the plasma concentration-time curves from time zero to infinity ($AUC_{t-\infty}$), elimination rate constant (β), half-life for the elimination phase ($t_{1/2\beta}$), clearance (Cl), the volume of distribution (Vdss) and the mean residence time (MRT). All data were expressed as mean ± standard derivation. The $AUC_{0-\infty}$ were determined by the addition of AUC_{0-t} calculated by the trapezoidal rule and $AUC_{t-\infty}$ calculated by extrapolate to time infinity. The β value was the terminal slope calculated by linear regression of the logarithmic value of the terminal phase. The Cl value was calculated as $Dose/AUC_{0-\infty}$. Area under the first moment curve (AUMC) was determined as $\int t \cdot C_p \cdot dt$. MRT was calculated as AUMC/AUC. The student's *t*-test statistical test was used to determine the difference among these data.

RESULTS AND DISCUSSION

The chromatograms of blank plasma and spiked plasma containing 10 ng/mL of CCK3 and 1 μg/mL of IS are presented in Figure 2A and B. The peaks are well separated. Figure 2C shows the chromatogram of extracted plasma obtained 5 min after IV administration of 3.97 mg/kg of CCK3. The compounds were well resolved without interference from biological unknown substances. For ascertainment the CCK3 peak was in fact not partly or largely due to a metabolite. The photodiode array detector was used to scan the drug peak. The result showed the chromatograms of standard drug and extraction from actual plasma sample were the same (data not shown). The retention time of CCK3 and IS were 6.5 and 3.8 min, respectively.

After several trials, it was found that the one-step liquid-liquid extraction of CCK3 in plasma was satisfactory. CCK3 was spiked in plasma at final concentrations of 0.05, 1 and 2 μg/mL, and then extracted by dichloromethane according the extraction procedure. The analyte recovery averaged 85.13 ± 4.65% (n = 3), suggesting that the extraction method was reproducible and suitable for the analysis of plasma samples.

A standard curve, with concentrations ranging from 10 to 3000 ng/mL in 0.2 mL of plasma samples, was constructed. The peak area of CCK3 was divided by the peak area of internal standard to obtain a peak area ratio. The data from the standard curves were analyzed using regression analysis to obtain the slope, intercept and correlation coefficient. The slopes, intercepts and correlation coefficients were 1.86 ± 0.04, -0.0006 ± 0.0456, and 0.9972 ± 0.1588 for intra-day analysis, whereas 1.92 ± 0.03, -0.0010 ± 0.0444, and 0.9975 ± 0.1546 for inter-day analysis, respectively (mean ± SE, n = 3). The correlation coefficients for intra- and inter-day were about 0.99, showing linearity between these concentration ranges. The lower limit of detection (LOD) for CCK3 under these conditions was 5.0 ng/mL. The sensitivity of electrochemical detector used was about 100 times of Ultraviolet (UV) absorbance measurement at 262 nm used (data not shown). According to earlier pharmacokinetic studies of amsacrine and its analogue[6], the lower limit of quantitation was 40 ng/mL. Therefore, the electrochemical detector was used to determine the CCK3 in plasma.

The precision expressed as the coefficients of variation (CV, %) was calculated for both intra- and inter-day analysis for each standard concentration. The accuracy was calculated as a relative error (RE, %). As shown in Table 1, all the CV and RE values were less than 7.69% and 8.16%, respectively, indicating that the assay was suitable for concentrations ranging from 10 to 3,000 ng/mL. The range was adequate for estimating plasma pharmacokinetics (Table 2 and Figure 3).

The drug plasma concentration-time profiles following intravenous administration of 3.972 and 7.944 mg/Kg CCK3 are shown in Figure 3. The plasma concentration-time profiles were analyzed using the PCNONLIN computer program. The correlation coefficient and AIC value were 0.9997 and -76.6 for dose of 3.972 mg/Kg, and 0.9965 and -64.9 for dose of 7.944 mg/Kg, respectively, suggesting that the plasma concentration-time profiles could be well described by two-compartment open model with first-order elimination process. The predicted concen-

Table 1. Intra- and inter-day precision (CV, %) and accuracy (RE, %) of determination of CCK3 in rat plasma

Expected	Intra-day			Inter-day		
concentration	Observed	C.V. (%)	R.E. (%)[a]	Observed	C.V. (%)	R.E. (%)
0.03 μg/mL	0.032 ± 0.003	7.69	6.66	0.031 ± 0.003	7.64	3.33
1.00 μg/mL	0.961 ± 0.047	4.95	-3.90	0.931 ± 0.046	4.94	-6.90
2.00 μg/mL	1.896 ± 0.026	1.37	-5.20	1.837 ± 0.025	1.37	-8.15

[a]R.E. (%) = (observed concentration–expected concentration)/expected concentration × 100%.

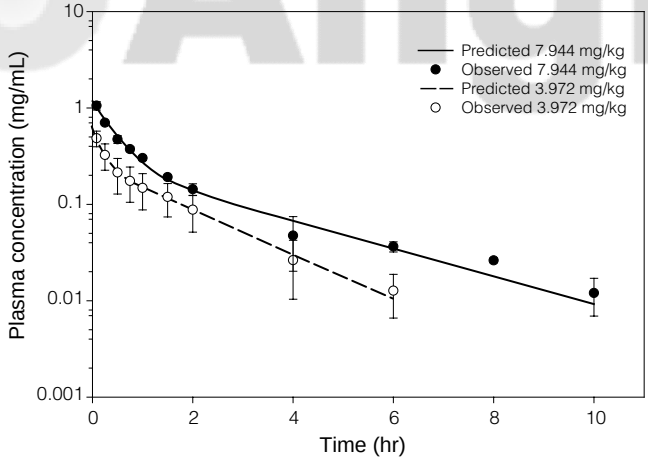

Figure 3. Plasma concentration-time curves of CCK3 after intravenous administration of 3.97 and 7.94 mg/kg in rats. Each point represents the mean ± S.D. (n = 3).

Table 2. Pharmacokinetic parameters of CCK3 after intravenous administration at doses of (3.97 and 7.94 mg/kg) in rats (n = 3)

Parameters		3.97 mg/kg	7.94 mg/kg
A	μg/mL	0.40 ± 0.33	0.90 ± 0.09
B	μg/mL	0.25 ± 0.10	0.37 ± 0.19
α	1/h	5.70 ± 1.75	3.37 ± 2.02
β	1/h	0.53 ± 0.11	0.46 ± 0.25
t1/2α	hr	0.13 ± 0.04	0.28 ± 0.15
t1/2β	hr	1.33 ± 0.24	1.72 ± 0.64
$AUC_{0-\infty}$	mg·hr/L	0.55 ± 0.20	1.12 ± 0.19[a]
$AUC_{0-\infty}$/Dose	mg·hr/L	0.14 ± 0.05	0.14 ± 0.01
Cl	L/hr	1.59 ± 0.53	1.44 ± 0.24
Vdss	L	2.61 ± 0.95	2.62 ± 0.76
MRT	ht	1.66 ± 0.26	1.89 ± 0.84

[a]Student-*t* test: significant difference ($p < 0.05$).

tration-time profiles are shown in Figure 3. The pharmacokinetic parameters are listed in Table 2. Comparing these parameters of CCK3 including half-life, volume of distribution and clearance, no significant difference was shown ($p > 0.05$) between these two doses (3.97 and 7.94 mg/kg). The average elimination half-life of CCK3 was 1.53 ± 0.28 hr, about 3 folds of amsacrine. From the plasma levels, the values of area under the plasma concentration-time curve ($AUC_{0-\infty}$) were 0.55 ± 0.20, and 1.12 ± 0.19 mg·hr/L, respectively. The $AUC_{0-\infty}$ increased proportionally with increase in the administration dose.

CONCLUSIONS

An HPLC method was developed to measure CCK3 in rat plasma. The method was used successfully in a preliminary study of the disposition of CCK3 in rats treated with dose of 3.972 and 7.944 mg/Kg. The plasma concentration of CCK3 versus time data was best fitted to a two-compartment open model with first-order elimination process and linear pharmacokinetics. The half-life of CCK3 in rat plasma was prolonged up to 1.53 ± 0.28 hr, about 3 folds of amsacrine. Therefore, CCK3 might have the potential to be tested clinically.

ACKNOWLEDGEMENTS

The authors are grateful to the National Science Council, Taiwan, for the financial support of this study (NSC 93-2320-B-037-047).

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