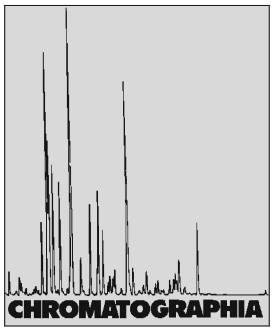
Highly Sensitive Quantitative Analysis of 1-[3-(Furo[3,2-*c*]quinolin-4-ylamino) phenyl]ethanone Oxime, a New Antitumor Agent, in Rat Plasma by LC with Electrochemical Detection



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Abstract

1-[3-(Furo[3,2-*c*]quinolin-4-ylamino)phenyl]ethanone oxime (CCK2) is an antitumor agent which was especially active against the growth of the renal cancer cell UO-31 ($GI_{50} = 0.03$ μM) and the two melanoma cancer cells UACC-257 ($GI_{50} < 0.01$ μM) and UACC-62 ($GI_{50} < 0.01$ μM) in cytotoxicity evaluation of NCI's full panel of 60 human cancer cell lines. From structure–activity relationships for amsacrine and CCK2, CCK2 is, moreover, expected to have a longer half-life than amsacrine in plasma. A sensitive high-performance liquid chromatographic method with electrochemical detection has therefore been developed and validated for determination of the pharmacokinetics of CCK2 in rats. Plasma samples were spiked with 2-naphthol as internal standard and extracted with dichloromethane. The analytes were separated on a C_{18} reversed-phase column (55 × 4 mm) with 20% acetonitrile, 5% tetrahydrofuran, and 75% pH 3.0 McIlvaine buffer as mobile phase at a flow rate of 1.0 mL min^{-1}. Electrochemical detection of CCK2 was performed at 1.0 V and 20 nA. Intra-day and inter-day precision and accuracy were acceptable down to the limit of quantification of 10 ng mL^{-1}. The lower limit of detection was 5 ng mL^{-1}. In an in vivo study pharmacokinetic data were determined for CCK2 in rat after intravenous administration of 6, 12, and 24 μmol kg^{-1}. The apparent volume of distribution, elimination half-life, and clearance were not significantly different among the three doses. The area under the plasma concentration–time curve increased in proportion to increasing dose. The elimination half-life of CCK2 was 3.4 times that of amsacrine. CCK2 might, therefore, have the potential to be tested clinically. The results also showed that this selective, sensitive, and reproducible LC method could be successfully applied to investigation of the pharmacokinetics of CCK2.

Keywords

Column liquid chromatography
Amsacrine
Antitumor
1-[3-(Furo[3,2-*c*]quinolin-4-ylamino)phenyl]ethanone oxime
Pharmacokinetics

Introduction

Amsacrine is a substituted 9-aminoacridine derivative which has clinical activity against acute lymphocytic and non-lymphocytic leukemias. Its relatively short half-life in plasma (~20–30 min) reduces its bioavailability, however,

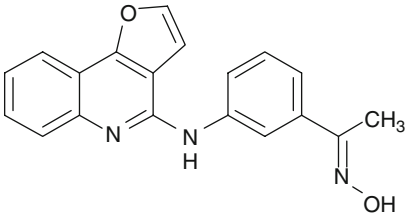

Fig. 1. The structure of 1-[3-(furo[3,2-*c*]quinolin-4-ylamino)phenyl]ethanone oxime (CCK2)

which limits its clinical application [1–4]. A series of structure–activity relationship (SAR) studies has therefore been conducted to obtain a drug with greater potential antitumor activity and longer half-life in human plasma [5–8]. The major route of breakdown of amsacrine and its analogs in vivo is nonenzymatically mediated attack of thiol at C(9), resulting, eventually, in loss of the side chain and formation of inactive products [9–12]. The furo[2,3-*b*]quinoline system, with higher electron density than that of acridine, was used to replace acridine, furnishing a 4-anilinofuro[2,3-b]quinoline derivative with longer half-life [8]. For the SAR studies a series of 4-anilinofuro[2,3-*b*]quinoline derivatives were synthesized and expected to have a longer half-life in plasma [12]. Among these derivatives, 1-[3-(furo[3,2-*c*]quinolin-4-ylamino)phenyl]ethanone oxime (CCK2; Fig. 1) was found to be especially active against growth of the renal cancer cell UO-31 ($GI_{50} = 0.03$ μM) and the two melanoma cancer cells UACC-257 ($GI_{50} < 0.01$ μM) and UACC-62 ($GI_{50} < 0.01$ μM) in evaluation of cytotoxicity against the NCI's full panel of 60 human cancer cell lines [12], indicating that CCK2 might have the potential to be tested clinically. To prove that the elimination half-life of CCK2 was longer than that of the original compound, a highly sensitive and robust analytical method had to be developed to measure the pharmacokinetic properties of CCK2. Analysis of amsacrine and its derivatives in plasma has been achieved by use of a non-specific fluorescence method, gas chromatography (GC), and LC with UV or electrochemical detection [13–17]. Our objective was to establish a sensitive, selective, and relatively rapid LC method which could be used for measurement of the pharmacokinetics of CCK2 in rats after administration of a dose as low as 6 μmol kg^{-1}.

Experimental

Chemicals, Reagents, and Solutions

1-[3-(Furo[3,2-*c*]quinolin-4-ylamino) phenyl]-ethanone oxime (CCK2) was synthesized in the laboratory of the School of Medicinal and Applied Chemistry (Kaohsiung Medical University, Taiwan). 2-Naphthol and tetraglycol were purchased from Sigma (St Louis, MO, USA). Methanol and acetonitrile were obtained from Tedia (Fairfield, OH, USA). Dichloromethane was purchased from Merck (Darmstadt, Germany). *N*,*N*-Dimethylacetamide was obtained from Mallinckrodt (Hazelwood, MO, USA). Other chemicals and solvents were of analytical-reagent grade.

Standard solutions of CCK2 were prepared in methanol. Calibration solutions were prepared in drug-free rat plasma spiked with standard solutions to furnish appropriate final concentrations ranging from 10 to 3,000 ng mL^{-1}. The internal standard solution (IS, 2-naphthol, 4 μg mL^{-1}) was prepared in methanol.

Apparatus and Chromatographic Conditions

LC was performed with an Hitachi model L-7100 pump, a Jasco 855-AS autosampler, a BAS LC-4C, and an Hitachi model L-4000H UV detector. A BAS MF 1000 dual glassy-carbon working electrode and a DAS RE6 Ag/AL reference electrode were used for electrochemical detection of CCK2. Compounds were separated on a 55 × 4 mm i.d., 3-μm particle, Merck LiChrocart reversed-phase C_{18} column. The mobile phase was a mixture of 20% acetonitrile, 5% tetrahydrofuran, and 75% pH 3.0 McIlvaine buffer consisting of 0.1 M disodium phosphate and 0.05 M citric acid (4.1:15.9, *v*/*v*); the flow rate was 1.0 mL min^{-1}. The column effluent was monitored by electrochemical detection at 1.0 V and 20 nA.

Preparation of Plasma Samples

Blank or spiked plasma, or plasma samples from rats after administration of CCK2 (0.2 mL) was mixed with 0.1 mL IS solution and 4 mL dichloromethane. The mixture was shaken horizontally for 50 min then centrifuged at 2,000 *g* for 10 min. The organic layer was transferred to another tube and evaporated to dryness under vacuum. The dry residue was reconstituted in methanol and 20 μL of the clear supernatant was injected for LC analysis.

Recovery of CCK2 from plasma was assessed by comparing peak areas obtained from samples spiked with CCK2 with those obtained from standards prepared in methanol and processed in the same way as samples. Plasma calibration plots in the range 10–3,000 ng mL^{-1} were prepared by similar processing of calibration solutions in drug-free rat plasma.

Replicate samples ($n = 5$) containing CCK2 at concentrations of 30, 1,000, and 2,000 ng mL^{-1} were processed on two separate days to evaluate intra-assay and inter-assay accuracy and precision. The limit of detection (LOD) of CCK2 was also determined in this analysis as the smallest concentration for which signals could be distinguished from the noise level at a typically acceptable signal-to-noise ratio of 3:1. In this study the lower limit of quantification was set at twice the LOD.

Determination of the Pharmacokinetics of CCK2

Wistar rats weighing 190–220 g (Laboratory Animal Center of the National Science Council) were used for the study. Rats were anesthetized by intraperitoneal injection of 25% urethane (3 mL kg^{-1}) then an aqueous solution of CCK2 was administered via the tail vein at a dose of 6, 12, or 24 μmol kg^{-1}. Blood samples were collected from the jugular vein at

appropriate times after administration. The blood samples were then centrifuged at 8,500 *g* for 10 min and the plasma was immediately separated and stored at −20 °C until analysis.

Analysis of Pharmacokinetic Data

Concentration–time profiles were analyzed by use of WinNonLin computer software (V4.0; Pharsight Software, Mountain View, CA, USA) to determine the type of compartment model by use of the correlation coefficient from observed and predicted data. The pharmacokinetic data measured were: area under plasma concentration–time curves from time zero to infinity ($AUC_{0-\infty}$), elimination rate constant (k), half-life of the elimination phase ($t_{1/2\beta}$), clearance (Cl), volume of distribution (V_{dss}), and mean residence time (MRT). All data were expressed as mean ± standard deviation. $AUC_{0-\infty}$ was calculated by use of the trapezoidal rule (AUC_{0-t}) and extrapolated to time infinity by addition of $AUC_{t-\infty}$. The k value was the terminal slope, which was calculated by linear regression of the logarithmic value of the terminal phase. The Cl value was calculated as dose/$AUC_{0-\infty}$ and V_{dss} was obtained by summation of central and tissue compartments. MRT was calculated from AUMC/AUC. Statistical analysis was performed by using ANOVA to determine differences among the data.

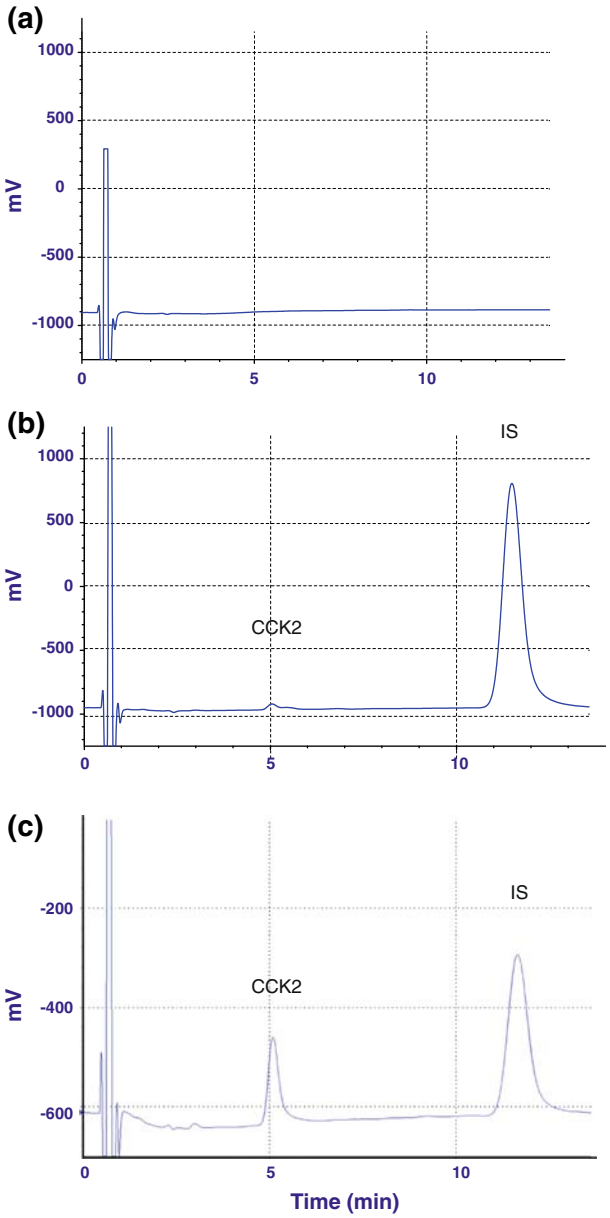

Fig. 2. LC chromatograms obtained from CCK2 in rat plasma: **a** blank plasma; **b** blank plasma spiked with CCK2 (10 ng mL^{-1}) and internal standard (IS, 4 ng mL^{-1}); **c** rat plasma extracted 1.5 h after iv administration of 12 μmol kg^{-1} CCK2

Results and Discussion

After a scan of potentials in the range 0.8–1.2 V, 1.0 V was chosen as the working potential. As illustrated in Fig. 2, LC separation on a C_{18} column with 20% acetonitrile, 5% tetrahydrofuran, and 75% pH 3.0 McIlvaine buffer as mobile phase at a flow rate of 1.0 mL min^{-1} with electrochemical detection at 1.0 V and 20 nA enabled separation of CCK2 and the internal standard without interference from biological impurities. The retention times of CCK2 and the IS were 5.4 and 12.1 min, respectively.

In previous studies [13–17] the organic solvents ether, dichloromethane, chloroform, and an ether–hexane mixture have been used to extract the CCK2 from plasma. The results showed that extraction with dichloromethane was most efficient and there was no interference from either blank plasma or alkaline plasma. Dichloromethane was therefore used as the optimum extraction solvent in this work. Recovery was determined for 50, 1,000, and 2,000 ng mL^{-1} CCK2 ($n = 5$) and found to be independent of analyte concentration. Average recovery was 97.00 ± 1.13%. Average recovery of the IS at 4 μg mL^{-1} was 98.05 ± 5.53%. These results showed that the method of extraction was reproducible and suitable for analysis of CCK2 in plasma.

A calibration plot in the range 10–3,000 ng mL^{-1} was constructed by analysis of 0.2-mL plasma samples. The

Table 1. Intra-day and inter-day precision (CV, %) and accuracy (RE, %) for analysis of CCK2 in rat plasma

Expected concentration	Intra-day			Inter-day		
	Observed	CV (%)	RE (%)	Observed	CV (%)	RE (%)
10 ng mL^{-1}	9.23 ± 0.71	7.39	7.78	9.12 ± 1.13	12.39	8.89
30 ng mL^{-1}	29.30 ± 3.45	11.52	3.33	31.11 ± 3.11	9.82	6.30
1.0 μg mL^{-1}	0.94 ± 0.04	4.71	6.12	0.96 ± 0.04	5.70	2.90
2.0 μg mL^{-1}	2.05 ± 0.10	4.69	2.40	1.90 ± 0.10	4.89	5.20
3.0 μg mL^{-1}	2.99 ± 0.05	1.80	0.44	2.99 ± 0.08	2.68	0.39

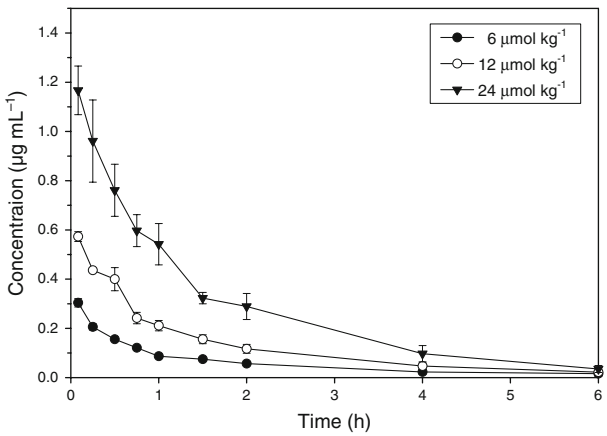

Fig. 3. Plasma concentration–time curves for CCK2 after intravenous administration of 6, 12, or 24 μmol kg^{-1} to rats ($n = 3$). Values are mean ± SD

peak area of CCK2 was divided by the peak area of the internal standard to obtain the peak-area ratio. Calibration data were treated by regression analysis to obtain the slopes, intercepts, and correlation coefficients. These were 0.3257 ± 0.0021, −0.0005 ± 0.0027, and 0.9997 ± 0.0095, respectively, for the intra-day calibration plot and 0.3259 ± 0.0023, −0.0002 ± 0.0030, and 0.9996 ± 0.0105, respectively, for the inter-day calibration plot. The typical intra-day and inter-day correlation coefficients (0.999) showed linearity was good in this concentration range. The lower limit of detection (LOD) for CCK2 under these conditions was 5.0 ng mL^{-1}. The LOD was approximately 60 times better than for ultraviolet absorbance detection (LOD = 300 ng mL^{-1}). In previous studies of the kinetics of amsacrine and its analogue, the lower limit of quantitation in plasma was approximately 20 ng mL^{-1} [16]. In this study it was necessary to increase the sensitivity of detection to determine pharmacokinetic data for CCK2 at lower doses than those of amsacrine. Electrochemical detection was therefore a suitable method for analysis of CCK2 in plasma.

Intra-day and inter-day precision, as coefficient of variation (CV, %), was calculated for all the calibration standards. Accuracy was calculated as relative error (RE, %). As shown in Table 1, all the CV and RE values were less than 12.39 and 8.89%, respectively. The result showed that plasma concentrations ranging from 10 to 3,000 ng mL^{-1} were appropriate for determination of the pharmacokinetics of CCK2 (Fig. 3).

Drug plasma concentration–time profiles obtained after intravenous administration of 6, 12, and 24 μmol kg^{-1} CCK2 are shown in Fig. 3. The plasma concentrations of CCK2 versus time data were fitted by WinNonLin computer software using different compartment models. The highest correlation coefficient ($r > 0.9967$) and lowest the AIC value (AIC < −65.18) were obtained by use of a two-compartment open model with first-order elimination processes, suggesting the plasma concentration–time profiles were best described by this model. The pharmacokinetic data for CCK2 were therefore calculated in accordance with this model. From Fig. 3 it is apparent that, in this model, the initial distribution was very rapid compared with the terminal phase. After administration of the three doses 6, 12, and 24 μmol kg^{-1} the respective pharmacokinetic data were 2.33 ± 0.81, 1.98 ± 0.26, and 1.79 ± 0.30 h for MRT; 2.14 ± 0.32, 2.09 ± 0.18 and 1.64 ± 0.03 L kg^{-1} for V_{dss}; 0.97 ± 0.19, 1.06 ± 0.12, and 0.76 ± 0.36 L h^{-1} for Cl; 1.91 ± 0.65, 1.66 ± 0.26, and 1.50 ± 0.36 h for $t_{1/2\beta}$; and 0.41 ± 0.08, 0.80 ± 0.09, and 1.66 ± 0.31 mg h L^{-1} for $AUC_{0-\infty}$. Comparison of these data revealed there were no significant differences ($P > 0.05$) among results obtained for MRT, V_{dss}, or Cl after the three different doses. $AUC_{0-\infty}$ increased in proportion to increasing dose administered. For $t_{1/2\beta}$, although there were no significant differences ($P > 0.05$) among the values obtained after the three doses, the average elimination half-life (1.69 ± 0.43 h) for CCK2 was approximately 3.4 times that for amsacrine. The pharmacokinetics of CCK2 were found to be linear over the dose range studied, however, and the results obtained provide a reasonable and objective rationale for clinical research.

Conclusion

A sensitive LC method has been developed for analysis of CCK2 in rat plasma. The method was used successfully in a preliminary study of the disposition of CCK2 in rats treated with 6, 12, or 24 μmol kg^{-1}. Plasma concentration–time data for of CCK2 were best fitted to a two-compartment open model with first-order elimination and linear pharmacokinetics. The elimination half-life of CCK2 in rat plasma was 1.69 ± 0.43 h, approximately 3.4 times that for amsacrine. CCK2 might, therefore, have the potential to be tested clinically.

Acknowledgments

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