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Short communication

Highly sensitive analysis of the anti-tumor agent 1-[4-(furo[2,3-b]-quinolin-4-ylamino)phenyl]ethanone in rat plasma by high-performance liquid chromatography using electrochemical detection

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Abstract

A sensitive high-performance liquid chromatography method with electrochemical detection was developed for the purpose of determining the concentration of the new anti-tumor agent 1-[4-(furo[2,3-b]-quinolin-4-ylamino)phenyl]ethanone (FQPE) in rats. The plasma samples were spiked with the internal standard diclofenac and extracted using dichloromethane. A C_{18} 250 mm × 4 mm column was used for the separation of analyte with a mobile phase consisting of 50% acetonitrile and 50% pH 3.0 of sodium 1-pentansulfonate solution at a flow rate of 1.0 mL/min. FQPE was detected by electrochemical detector at 1.0 V and 20 nA. Intra-day and inter-day precision and accuracy were acceptable down to the limit of quantization of 1 ng/mL. The lower limit of detection (LOD) was 0.5 ng/mL. The pharmacokinetic parameters of FQPE in rats after intravenous administration of 2.1 and 4.2 mg/kg were determined. The apparent volume of distribution, half-life of elimination, and clearance showed no significant difference between the two dosages. The area under the plasma concentration time curve increased proportionally with dose. The half-life of FQPE was prolonged about 2.4-fold, compared with amsacrine.

Keywords: Amsacrine; 1-[4-(furo[2,3-b]-quinolin-4-ylamino)phenyl]ethanone; Anti-tumor; Pharmacokinetics; Half-life

1. Introduction

Amsacrine is a substituted 9-aminoacridine derivative that has clinical activity against acute lymphocytic and non-lymphocytic leukemias [1–5]. But the half-life of amsacrine in plasma is very short, about 20–30 min, resulting in limited bioavailability in clinical use [6]. Therefore, a series of amsacrine analogs such as DNA-intercalating 9-anilinoacridine derivatives were designed and synthesized for prolonging the half-life [7–9]. Su et al. reported that 3-(9-acridinylamino)-5-(hydroxymethyl)aniline (AHMA) had potent anti-tumor activity against leukemic L1210 and HL-60 both in vitro and in vivo, and long half-life in human plasma [7]. The major route of breakdown for amsacrine and its analogs in vivo is a non-enzymatically mediated attack of thiol at C(9), resulting eventually in loss of the side chain and the formation of inactive products [10–12]. Some studies [13,14] reported that the furo[2,3-b]quinoline ring system is a constituent of an important group of bioactive natural products such as dictamine, robustine, and haplopine, and possesses a higher electron density than that of acridine. Therefore, the metabolic rate possibly might be lowered by replacing the acridine with the furo[2,3-b]quinoline ring moiety. In previous studies, a series of 4-anilino furo[2,3-b]quinoline derivatives have synthesized and were

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expected to have longer half-life of elimination in plasma [15]. Among these derivatives, 1-[4-(furo[2,3-b]-quinolin-4-ylamino)phenyl]ethanone (FQPE) (GI_{50} = 0.025 μM) was found to exhibit superior anti-tumor activity compared with both clinically used anticancer drugs amsacrine (GI_{50} = 0.44 μM) and daunomycin (GI_{50} = 0.044 μM) in the NCI's full panel of 60 human cancer cell lines cytotoxicity evaluation [15]. In order to prove our hypothesis that FQPE has longer half-life, a highly sensitive and robust method was developed for determining the pharmacokinetics of FQPE in rats following intravenous administration.

2. Experiment

2.1. Materials and reagents

1-[4-(furo[2,3-b]-quinolin-4-ylamino)phenyl]ethanone (Fig. 1) (FQPE) was synthesized in the laboratory of the School of Medicinal and Applied Chemistry (Kaohsiung Medical University, Taiwan). Diclofenac sodium and tetraglycol were purchased from Sigma (St. Louis, MO, USA). Methanol and acetonitrile were obtained from Tedia Co. (USA). Sodium salt of 1-pentanesulfonic acid was obtained from Wako (Japan). Dichloromethane was purchased from Merck (Germany). *N*,*N*-dimethylacetamide was obtained from Mallinckrodt (USA). All other chemicals and solvents were of analytical reagent grade.

2.2. Apparatus and chromatographic conditions

The high-performance liquid chromatography (HPLC) system consisted of a Hitachi model L-700 pump, a BAS LC-4C electrochemical detector, a Jasco 855-AS autosampler, and a Merck Lichrocart® C_{18} column (250 mm × 4 mm I.D., 5 μm). The mobile phase was a mixture of 50% acetonitrile and 50% 0.01 M sodium salt of 1-pentansulfonic acid solution (v/v, pH 3.0), and the flow rate was 1.0 mL/min. The effluent from the column was monitored by electrochemical detector at 1.0 V and 20 nA. The BAS MF 1000 dual glassy-carbon working electrode and DAS RE6 Ag/AL reference electrode were used.

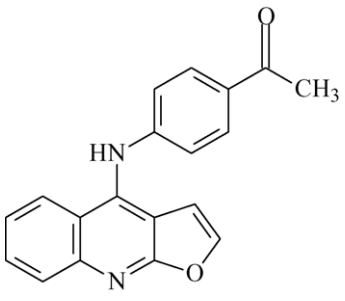

Fig. 1. Structures of 1-[4-(furo[2,3-b]-quinolin-4-ylamino)phenyl]ethanone (FQPE.).

2.3. Drug standards

Standard solutions of FQPE were prepared in methanol. Standard samples of plasma were prepared using drug-free rat plasma spiked standard solution to obtain the appropriate final concentrations ranging from 1 to 15,000 ng/mL. The internal solution (IS, diclofenac sodium, 6 μg/mL) was prepared in methanol.

2.4. Preparation of plasma samples

Aliquots of blank, spiked or plasma samples 0.2 mL were mixed with 0.1 mL of IS solution and 4 mL of dichloromethane. The mixture was horizontally shaken for 30 min and centrifuged at 3000 rpm for 10 min at ambient temperature. The 3 mL of organic layer was transferred to another tube and evaporated to dryness by vacuum pump. The dry residue was reconstituted in 0.2 mL acetonitrile and the 20 μL of clear supernatant was injected into the HPLC system.

The recovery of FQPE from plasma was assessed by comparing the peak area obtained from spiked FQPE samples with those standards in acetonitrile solution and processed as per samples.

Standard samples of rat plasma were prepared and analyzed during method validation. Linearity of standard curves, intra-day and inter-day precision and accuracy were determined from these data. The limit of detection (LOD) of FQPE was determined. LOD is the smallest concentration at which signals can be distinguished from the noise level; a typically acceptable signal-to-noise ratio is 3:1.

2.5. Determination of FQPE pharmacokinetics

Wistar rats weighing 180–250 g were used in the study (Laboratory Animal Center of the National Science Council, Taiwan). Rats were anesthetized by intraperitoneal injection of 25% urethane (3 mL/kg). A 0.5 mL injection of FQPE aqueous solution was administered via the tail vein at dosages of 2.1 and 4.2 mg/kg. Blood samples of 0.45 mL were collected from the jugular vein at appropriate time intervals after FQPE administration. The blood samples were then centrifuged at 12,000 rpm for 10 min, and plasma was immediately separated and stored at −20 °C until analysis.

2.6. Analysis of pharmacokinetic data

Concentration–time profiles were analyzed using the WinNonLin computer program (V4.0, Pharsight Software, USA). The following pharmacokinetic parameters were assessed: the area under the plasma concentration-time curves from time zero to infinity ($AUC_{0-\infty}$), elimination rate constant of terminal phase (β), half-life of elimination ($t_{1/2\beta}$), clearance (Cl), the volume of distribution (V_{dss}), and the mean residence time (MRT). All data were expressed as mean ± S.D. The $AUC_{0-\infty}$ were determined by the addition of AUC_{0-t}

calculated by the trapezoidal rule and $AUC_{t-\infty}$ calculated by extrapolated to time infinity. The β 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/$AUC_{0-\infty}$. Area under the first moment curve (AUMC) was determined as $\int tC_p\,dt$. MRT was calculated as AUMC/AUC. Statistical analysis was performed using Student's *t*-test to determine the difference among these data.

3. Results and discussion

To develop a rapid and sensitive HPLC method with electrochemical detection for the determination of FQPE in plasma samples, the hydrodynamic voltammogram of compound was performed in order to select the optimum potential value for the detection. For this purpose, a potential scan in the range of 0.8 to 1.2 V was applied to a solution. The mean value of peak area obtained was plotted versus potential as shown in Fig. 2. Potential values reached to 1.0 V produce a stable response. A potential of 1.0 V was chosen as the working potential. The mobile phase composition is critical for the method selectivity, too. To optimize separation of FQPE from endogenous plasma compounds, the current HPLC procedure with a mobile phase consisting of 50% acetonitrile and 50% pH 3.0 of sodium salt of 1-pentansulfonic acid solution (v/v) at a flow rate of 1.0 mL/min was used. Chromatograms of blank plasma and spiked plasma containing 4 ng/mL of FQPE and 6 μg/mL of IS are presented in Fig. 3A and B. The retention time of FQPE and IS were 3.6 and 7.4 min, respectively. Fig. 3C shows a chromatogram of extracted plasma obtained 0.5 h after administering FQPE 4.2 mg/kg. The compounds were well resolved and there was no interference from biological impurities. For ascertainment that the FQPE peak is in fact not partly or largely due to a metabolite, the photodiode array detector was used to scan peak of the drug. The result showed the chromatograms of standard drug and those extracted from actual plasma sample were same.

After several trials, it was found that the one-step liquid-liquid extraction of FQPE in plasma was satisfactory. The recovery of analyte was not dependent on the analyte concentration and averaged 88.94 ± 6.81%, showing that the extraction method was reproducible and suitable for the analysis of plasma samples.

Two standard calibration curves, ranging from 1 to 250 ng/mL and from 0.06 to 15 μg/mL were constructed. The peak area of FQPE was divided by the peak area of internal standard to attain a peak area ratio. The data from the standard curves were analyzed using regression analysis to obtain the

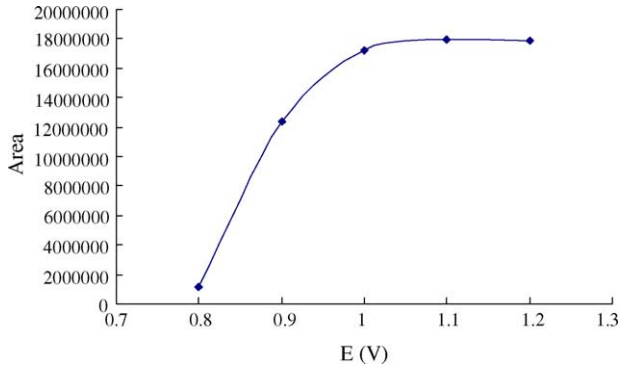

Fig. 2. Hydrodynamic voltammogram of FQPE (1 μg/mL).

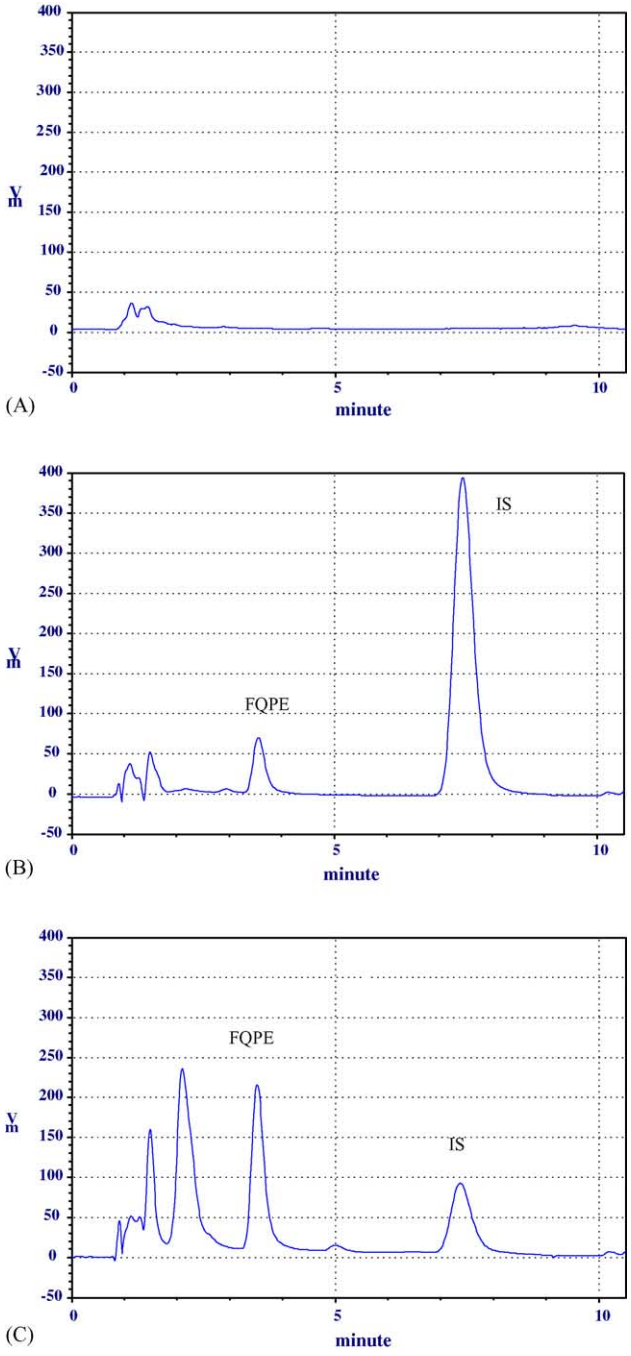

Fig. 3. HPLC chromatograms of FQPE in rat plasma. (A) Blank plasma; (B) plasma spiked with FQPE (4 ng/mL) and internal standard (6 μg/mL); (C) extracted rat plasma 30 min after IV administration of 4.2 mg/kg.

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

Expected (μg/mL)	Intra-day			Inter-day		
	Observed	C.V. (%)	R.E. (%)	Observed	C.V. (%)	R.E. (%)
0.0025	0.0023 ± 0.0003	7.88	12.1	0.0023 ± 0.0003	5.22	12.1
0.05	0.0508 ± 0.0030	1.66	5.93	0.0528 ± 0.0010	5.66	1.62
10.0	10.0815 ± 0.5745	0.81	5.70	9.8262 ± 0.2959	1.74	3.01

R.E. (%) = (observed concentration − expected concentration)/expected concentration × 100%.

slopes, intercepts, and correlation coefficients. The slopes, intercepts, and correlation coefficients were 9.108 ± 0.183, 0.001 ± 0.021, and 0.9974 ± 0.0667 for the lower concentration range, as well as 9.133 ± 0.064, −0.066 ± 0.444, and 0.9997 ± 1.3950 for the higher concentration range, respectively (mean ± S.E., $n = 3$). The correlation coefficients for lower and higher concentration ranges of calibration curves were typically 0.99, showing that there was good linearity between these concentration ranges. The LOD was 0.5 ng/mL. The sensitivity of the electrochemical detector used was about 100 times of ultraviolet (UV) absorbance measurement at 380 nm used (data not shown). According to earlier pharmacokinetic studies of amsacrine and its analogue [16], the lower limit of quantitation is 40 ng/mL. Therefore, the electrochemical detector was optimally used to determine the FQPE in plasma.

The precision expressed as the coefficients of variation (CV, %) was calculated for both intra-day and inter-day 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 12.1% and 7.8%, respectively. It was indicted that the plasma assay was suitable for concentrations ranging from 1 to 15,000 ng/mL. The range was adequate for estimating plasma level in rats from a pharmacokinetic study (Table 2 and Fig. 4).

The drug plasma concentration-time profiles following intravenous administration of 2.1 and 4.2 mg/kg FQPE are shown in Fig. 4. The dose of administration was calculated according to the animal study of amsacrine [16]. The 4.2 mg/kg of FQPE was equivalent to 14 μmol/kg, which was approximate to the lowest dose of amsacrine. The plasma concentration of FQPE versus time data were best fitted by a two-compartment open model with first-order elimination processes. The initial distribution was very rapid compared with the terminal phase. The various pharmacokinetic parameters

Table 2
Pharmacokinetic parameters of FQPE after intravenous administration at dosages of 2.1 and 4.2 mg/kg in rats

Parameters		2.1 mg/kg ($n = 4$)	4.2 mg/kg ($n = 3$)
$AUC_{0-\infty}$/dose	μg h/L	80.95 ± 9.52	78.57 ± 2.38
$t_{1/2\beta}$	h	1.22 ± 0.17	1.11 ± 0.28
Cl	L/h	2.82 ± 0.37	2.80 ± 0.17
V_{ss}	L	3.41 ± 0.25	2.52 ± 0.34
MRT	h	1.22 ± 0.12	0.90 ± 0.12

*Significant difference ($p < 0.05$).

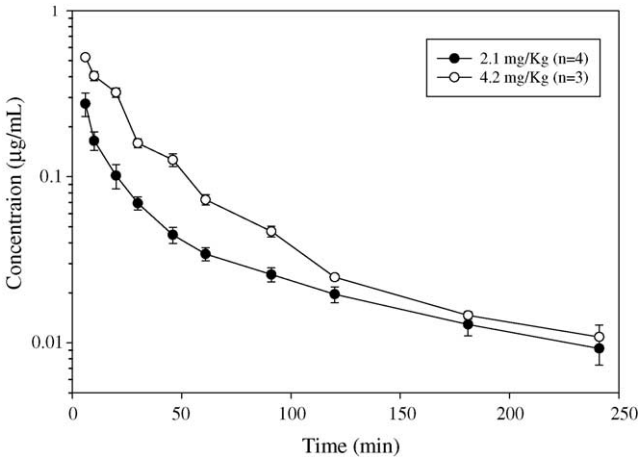

Fig. 4. Plasma concentration–time curves of FQPE after intravenous administration of 2.1 and 4.2 mg/kg in rats (mean ± S.D.).

of FQPE were estimated as shown in Table 2. Following the two different dosages, the average elimination half-life of FQPE was about 1.2 h. It was longer than amsacrine (about 0.5 h) [6], demonstrating our forecast that the metabolic rate of amsacrine could be lowered by replacing the acridine with the furo[2,3-b]quinoline ring moiety. Comparing these parameters of FQPE including half-life of elimination, volume of distribution, clearance, and $AUC_{0-\infty}$/dose, no significant differences were shown ($P > 0.05$) between these two dosages (2.1 and 4.2 mg/kg). It was represented that the linear relationship of pharmacokinetics between the doses of FQPE was found and provided to the design of additional pre-clinical studies.

4. Conclusion

A sensitive HPLC method with electrochemical detection was developed to measure FQPE in rat plasma. The method was used successfully in a preliminary study of the disposition of FQPE in rats treated with dosages of 2.1 and 4.2 mg/kg. The plasma concentration of FQPE versus time data were best fitted to a two-compartment open model with first-order elimination process and following linear pharmacokinetics. The elimination half-life of FQPE in rat plasma was prolonged about 2.4 fold compared with amsacrine.

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