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Short communication

Determination of the anticancer agent CI-980 in plasma by achiral liquid chromatography on a Pirkle-type stationary phase

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Abstract

A high-performance liquid chromatographic assay has been developed and validated for the determination of the anticancer agent CI-980 in plasma samples from pediatric patients. After ether extraction from alkaline plasma, the CI-980 was chromatographed on a (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine stationary phase using a mobile phase composed of hexane–isopropanol (70:30, v/v) modified with 1% acetonitrile. The method was linear over a 0.25 to 25.00 ng/ml range and the intra- and inter-day coefficients of variation (C.V.s) were less than 15%. The method was applied to the determination of the plasma concentration–time profile for a pediatric patient receiving 3.5 mg/m^2 of CI-980 per day as a continuous 72-h infusion.

Keywords: CI-980

1. Introduction

CI-980 is a novel anticancer agent which has potent antitubulin activity. CI-980 appears to bind to the colchicine binding site on tubulin and disrupts microtubule function by inhibiting tubulin polymerisation [1].

CI-980 is a chiral compound which is administered as a single enantiomer (*S*)-(5-amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazin-7-yl)-carbamic acid ethyl ester, Fig. 1A. The (*S*)-isomer has been shown to be a more potent antimitotic compound than the (*R*)-isomer and the two isomers differ markedly in binding to tubulin [2].

A high-performance liquid chromatographic assay has been previously developed and validated for human plasma to support Phase I clinical trials in adults [3]. This assay utilised solid-phase extraction followed by chromatography on an octadecylsilane stationary phase. This method proved to be irreproducible in our laboratory. Therefore, a new method was developed and validated for use with a Phase I clinical trial conducted by the Pediatric Oncology Group.

The initial problem with the previously reported assay was an extremely low recovery of CI-980 from plasma using solid-phase extraction. Varying the extraction matrix and the extraction solvents did not markedly improve the results. An alternative liquid–liquid extraction was developed with reasonably better results (>65% recovery) and reproducibility. However, the chromatogram of the extract on the octadecylsilica stationary phase contained an endogenous compound which coeluted with CI-980. A

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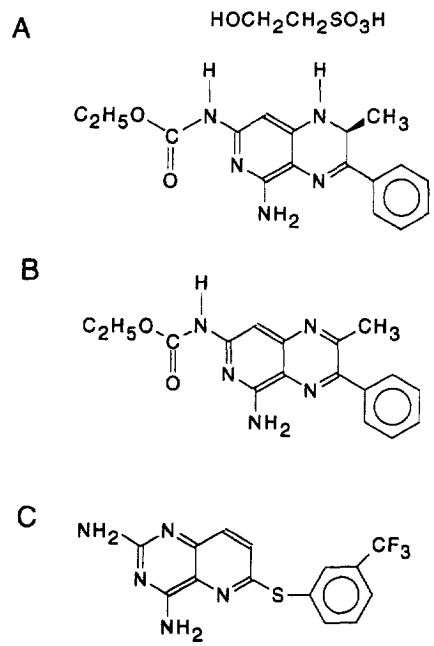

Fig. 1. Structures of the compounds used in this study: A=CI-980; B=CI-980 metabolite; C=internal standard.

variety of standard reversed-phase and normal-phase supports and chromatographic conditions were employed without successfully resolving CI-980 from the interference.

A reconsideration of the molecular structure of CI-980 suggested that this compound contains complimentary interaction sites relative to the (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine stationary phase. The predicted retention mechanism would include dipole–dipole and hydrogen bonding interactions between the carbamate ester on solute and the amide moiety of the stationary phase. In addition, $\pi-\pi$ interactions were possible between the heterocyclic portion of CI-980 and the dinitrobenzoyl group on the stationary phase. Similar solute–stationary phase interactions have been described for enantioselective separations observed on the stationary phase [4].

This manuscript reports the successful application of the (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine stationary phase to the quantitative determination of CI-980 in plasma. The resulting assay has been used to analyse plasma samples from pediatric patients receiving a continuous 72-h infusion of CI-980. Since the objective of the study was the pharmacokinetics of CI-980, the method was developed to separate CI-980 from its metabolite but not to quantify the metabolite.

2. Experimental

2.1. Chemicals

CI-980 {(*S*)-(5-amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazin-7-yl)carbamic acid ethyl ester 2-hydroxyethanesulfonate (1:1); Fig. 1A}, PD 080658 internal standard (I.S.) {6-[[3-(trifluoromethyl)phenyl]thio] - pyrido[3,2]pyrimidine-2,4-diamine; Fig. 1B} and PD 132182 metabolite (M) {(5-amino-2-methyl-3-phenylpyrido[3,4-*b*]pyrazin-7-yl)-carbamic acid ethyl ester; Fig. 1C} were provided by Parke-Davis Pharmaceutical Research (Ann Arbor, MI, USA). Acetonitrile, hexane, isopropanol and anhydrous ethyl ether were purchased from Fisher Scientific (Montreal, Canada). Sodium hydroxide was purchased from BDH (Ville St. Laurent, Canada). Pooled control human plasma was supplied by the Blood Bank of the Royal Victoria Hospital (Montreal, Canada).

2.2. Apparatus

HPLC was carried out using a Spectra-Physics P2000 pump (Spectra-Physics, San Jose, CA, USA) and a Spectra-Physics AS3000 autosampler equipped with a 500 μl loop. The solutes were detected using a Shimadzu RF-535 fluorescence detector (Fisher Scientific, Montreal, Canada) with $\lambda_{excitation}$=370 nm and $\lambda_{emission}$=475 nm. Data collection was carried out using a Spectra-Physics Chromjet integrator interfaced with a Spectra 386 computer equipped with Winner on Windows software for data collection. A Bakerbond (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine (DNBPG) covalently bonded 5 μm stationary phase packed in a 250×4.6 mm column (J.T. Baker, Phillipsburg, NJ, USA) and a 5 μm nitrile guard cartridge (Regis, Morton Grove, IL, USA) were used to carry out the chromatography.

2.3. Chromatographic procedures

The chromatographic separation of CI-980, its metabolite (M) and the internal standard I.S. was achieved with a mobile phase consisting of hexane–isopropanol (70:30, v/v) modified with 1% acetonitrile. A flow-rate of 1.0 ml/min and ambient temperature were used throughout the study.

2.4. Stock solutions

Stock solutions of CI-980 (500 μg/ml), M (250 μg/ml) and I.S. (400 μg/ml) were prepared in methanol and stored at −80°C. Stock plasma solutions were prepared containing 0.25, 0.50, 1.00, 2.00, 2.50, 5.00, 10.00, 12.50, 20.00 and 25.00 ng/ml of CI-980 solution. Similarly a solution of 15 ng/ml I.S. in methanol was prepared. All the standards were protected from light by wrapping tubes in foil or using amber vials.

2.5. Sample preparation

Aliquots (1 ml) of the appropriate CI-980 spiked stock plasma solutions were placed in culture tubes and 100 μl of I.S. solution (15 ng/ml) was added. The solution was vortex-mixed, 0.5 ml of 5 *M* sodium hydroxide added and the resulting solution vortex-mixed for 1 min. Ether (3.0 ml) was added, the mixture vortex-mixed for 1 min, centrifuged at 3000 *g* for 15 min, the tubes were placed in a dry ice–acetone bath for 20 min (until the aqueous phase was frozen) and the organic phase was decanted into a clean polypropylene tube. The ether was evaporated to dryness in a Speed-Vac concentrator (Savant Instruments, Farmingdale, NY, USA) and the residue was reconstituted using 250 μl of the mobile phase.

2.6. Validation studies

2.6.1. Standard curve

Triplicate standard curves were prepared by spiking drug-free plasma with known amounts of CI-980 and I.S. prior the extraction procedure. Concentrations of CI-980 were 0.50, 1.00, 2.50, 5.00, 12.50 and 25.00 ng/ml. Calibration curves plotting CI-980/I.S. peak-area ratios as a function of CI-980 plasma concentration were derived.

2.6.2. Recovery

The recovery of CI-980 was determined using spiked plasma at 2.00, 10.00 and 20.00 ng/ml. Recoveries were estimated by comparing the mean peak areas of extracted spiked plasma samples to the mean peak areas of equivalent aqueous standard solutions.

2.6.3. Accuracy and precision

Inter-day and intra-day variability studies were performed by spiking drug-free plasma with 2.00, 10.00 and 20.00 ng/ml CI-980. Five samples at each concentration were extracted on each of four consecutive days.

2.7. Clinical samples – Pediatric Oncology Group Phase I Study No. 9470

A Phase I trial of CI-980 was carried out in children with solid tumors. A dosage of 3.5 mg/m^2 per day was administered as a continuous 72-h infusion to patients. Plasma samples were obtained at hours 0 (pretherapy), 48 (during infusion), 72 (end of infusion), 72.5, 73, 74, 76, 80 and 96 (1/2, 1, 2, 4, 8 and 24 h post completion of infusion). The plasma was frozen at −80°C until assayed.

3. Results and discussion

3.1. Chromatographic results

Under the chromatographic conditions used in this study CI-980, M and I.S. were resolved (Fig. 2) and blank plasma samples were free of endogenous materials eluting at the same retention times as CI-980, M and I.S. (Fig. 3). The endogenous material was successfully resolved from CI-980 and was found to elute near the solvent front. Since the objective of this study was determination of CI-980 pharmacokinetics, the quantitation of M was not pursued; it was adequate to demonstrate that M did not interfere with the determination of CI-980.

Chromatograms resulting from the analysis of blank plasma and plasma spiked with CI-980 and internal standard are shown in Fig. 3A and B, respectively. Standard curves for CI-980 were linear over the range investigated with a regression equa-

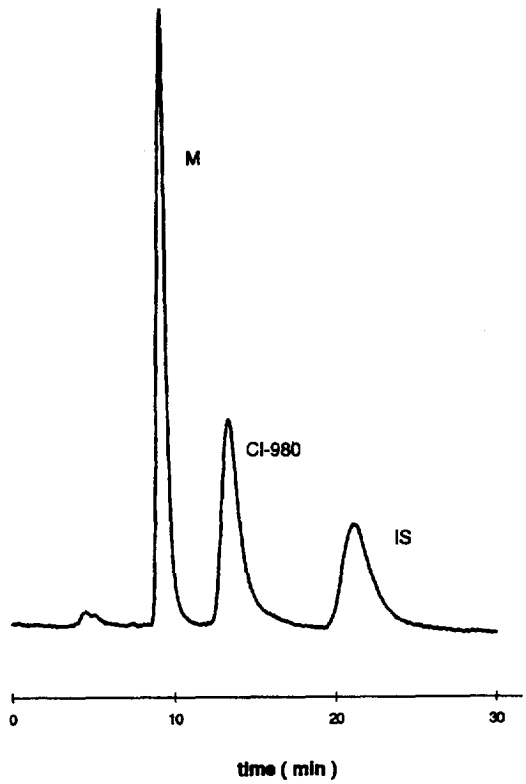

Fig. 2. Chromatographic separation of 67 ng/ml CI-980, 1000 ng/ml internal standard (I.S.) and 13 ng/ml CI-980 metabolite (M).

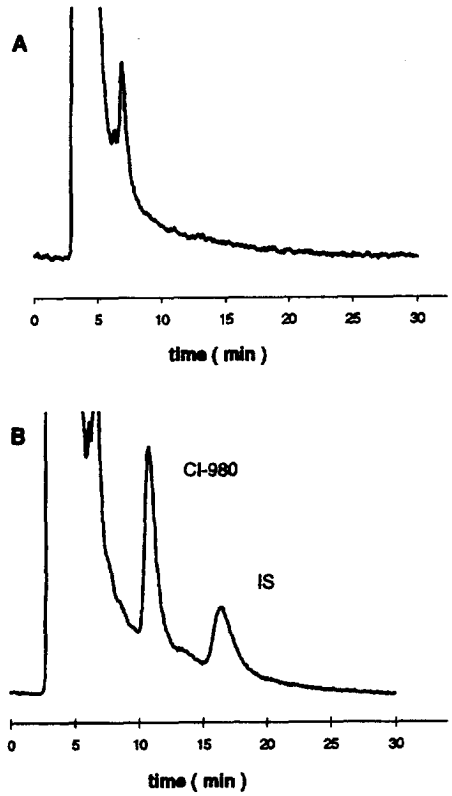

Fig. 3. Chromatograms of the analysis of extracted plasma samples: A=blank plasma; B=plasma spiked with 25 ng/ml CI-980.

tion and correlation coefficient of: $y=0.1872x-0.0493$, $r=0.999$.

The intra-day and inter-day variabilities are presented in Tables 1 and 2. In all cases the coefficient of variation (C.V.) were less than 15%. Recoveries for both CI-980 and I.S. exceeded 65%; CI-980: 67.2±2.1% ($n=5$) and I.S.: 73.3±1.8% ($n=5$).

3.2. Limits of detection and quantitation

The limit of detection (LOD) of CI-980 in human plasma was 0.25 ng/ml. The limit of quantification (LOQ) of CI-980 was 0.50 ng/ml.

3.3. Assay of patient samples

Chromatograms of plasma samples obtained from a pediatric patient who received a 3.5 mg/m^2 per day dose of CI-980 administered as a continuous 72-h infusion are presented in Fig. 4A–D. The plasma concentration–time profile of this patient is presented in Fig. 5.

Acknowledgments

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Table 1
Intra-day variabilities for the assay of CI-980 in plasma

Level CI-980 (ng/ml)	Mean observed concentration (ng/ml)	n	Standard deviation	C.V. (%)
2.00	1.80	5	0.09	5.0
10.00	10.60	5	0.48	4.5
20.00	22.90	5	1.65	7.2

Table 2
Inter-day variabilities for the assay of CI-980 in plasma

Level CI-980 (ng/ml)	Mean observed concentration (ng/ml)	n	Standard deviation	C.V. (%)
2.00	1.90	20	0.19	9.7
10.00	9.90	20	1.27	12.9
20.00	22.10	20	2.69	12.2

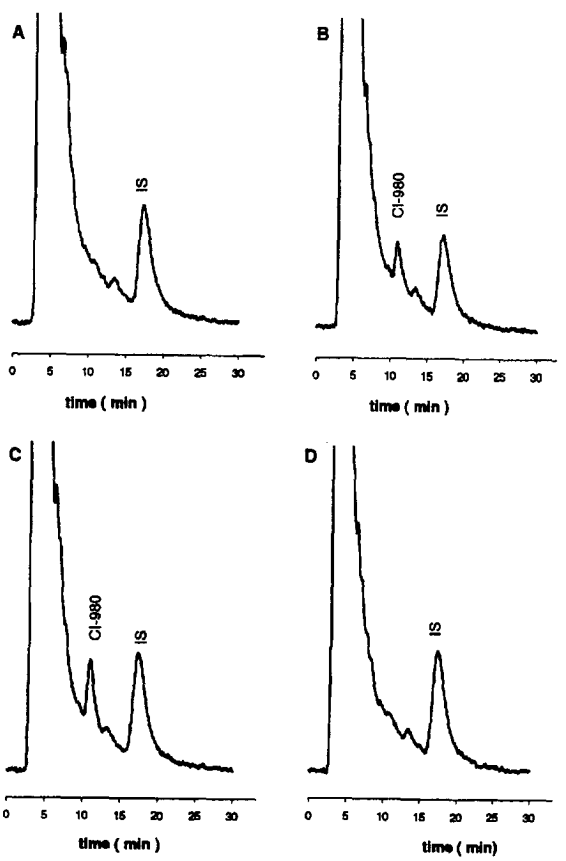

Fig. 4. Chromatograms from the analysis of plasma samples from a pediatric patient receiving 3.5 mg/m^2 of CI-980 per day as a continuous 72-h infusion: A=pretherapy (0 h); B=during infusion (48 h); C=end of infusion (72 h); D=24 h post completion of infusion (96 h).

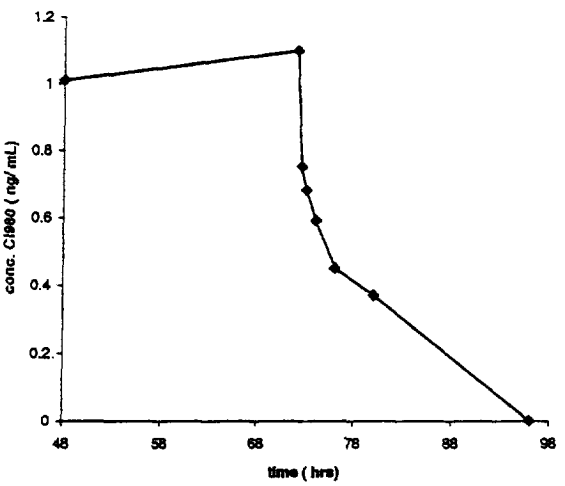

Fig. 5. Plasma concentration–time profile for a pediatric patient receiving 3.5 mg/m^2 of CI-980 per day as a continuous 72-h infusion.

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