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High-performance liquid chromatographic measurement of the novel anti-HIV agent 7,8-dihydrocostatolide (NSC 661123)

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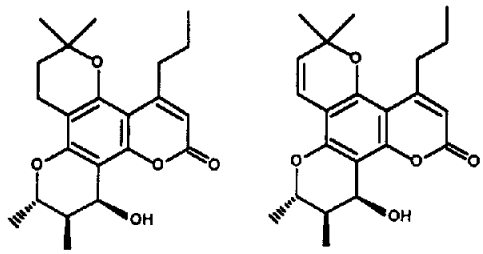
Abstract

An HPLC assay was used to determine levels of a promising anti-HIV agent 7,8-dihydrocostatolide (DC; NSC 661123) in human and murine plasma. The structurally related compound costatolide (C) was found to be a suitable internal standard. Drug was extracted from human or murine plasma using a solid-phase C_{18} cartridge. The compound was eluted from an ODS analytical HPLC column using an acetonitrile–water mobile phase. Drug was quantified over the assay range of 19.5 to 625 ng/ml with excellent within- and between-day reproducibility. Data resulting from the use of the assay method for determination of dihydrocostatolide pharmacokinetics in mice are presented.This is the first report of a validated HPLC assay for determining DC levels in human and mouse plasma.

1. Introduction

Costatolide (Fig. 1) has been isolated and identified from extracts of latex of the Malaysian plant *Calophyllum teysmannii* var. *ionphylloide* as part of a National Cancer Institute sponsored program to identify natural product compounds with anti-HIV activity. While the structure of costatolide related compounds has been known since 1964 [1], discovery of the anti-HIV activity of this class of compounds is a recent event [2]. The 7,8-dihydro derivative of costatolide (dihydrocostatolide; Fig. 1) was prepared by the NCI Laboratory of Drug Discovery Research and Development as a means of removing a potential site for *in vivo* oxidative metabolism and thus improving the compound's pharmacokinetic and pharmacodynamic activity. In anticipation of clinical testing of this compound, an analytical assay was developed which would be



Dihydrocostatolide Costatolide

Fig. 1. Structures of costatolide (C, NSC 661122) and 7,8-dihydrocostatolide (DC, NSC 661123).

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suitable for evaluation of pharmacokinetic parameters of dihydrocostatolide. Here we present a validated HPLC method for the determination of 7,8-dihydrocostatolide in human and mouse plasma. Also presented is the application of this method toward determination of murine derived pharmacokinetic data.

2. Experimental

2.1. Reagents and chemicals

Costatolide and 7,8-dihydrocostatolide were provided as dry powders by the National Cancer Institute. Acetonitrile used in the mobile phase was of analytical grade and was obtained from EM Science (Gibbstown, NJ, USA). Water was obtained daily from a Milli-Q system (Millipore, Bedford, MA, USA). Outdated human plasma was obtained from the M.D. Anderson Cancer Center Blood Bank (Houston, TX, USA). Mouse plasma was purchased from Pel-Freeze (Rogers, AR, USA).

Stock solutions of DC and C (1 mg/ml) were prepared in 100% acetonitrile and stored at −20°C. Under these conditions the solutions were stable for at least 1 month.

2.2. Extraction procedure

A 25-μl volume of a stock solution of internal standard (C at 25 μg/ml) was added to 1 ml of each plasma sample to be analyzed. After vortex-mixing, the sample was loaded onto a conditioned C_{18} Sep-Pak solid-phase extraction cartridge (Millipore, Bedford, MA, USA). The cartridges were then washed with 5 ml of water and the eluate discarded. Drug was eluted with 2 ml of ethyl acetate and the eluants dried under nitrogen. Samples were reconstituted in 250 μl of 100% acetonitrile and transferred to an automated injector vial for HPLC analyses.

2.3. Calibration curve and linearity

Calibration curves were prepared by spiking normal human or mouse plasma with increasing amounts of DC. The linearity in each of these matrices was assessed with concentrations of DC ranging from 19.5 to 625 ng/ml (19.5, 39, 78, 156, 312 and 625 ng/ml). The ratios of the peak areas for DC to C were plotted against the DC concentration to check for linearity, and the correlation coefficient was calculated.

2.4. Chromatography

The isocratic liquid chromatograph (Waters Assoc., Div. of Millipore, Millford, MA, USA) consisted of a Model 510 solvent delivery pump, a Model 717 autosampler and a Model 990 photodiode array detector. A wavelength of 318 nm was used.

Chromatographic separations were performed on a 250 × 4.6 mm I.D. Spherisorb ODS analytical column, 5 μm particle size (Phenomenex, Torrence, CA, USA). DC was eluted using a mobile phase of 80% acetonitrile in water. No addition of buffer or adjustment of pH was necessary. The flow-rate was 1.2 ml/min.

2.5. Detection limit

The limit of detection was defined as the lowest concentration of DC resulting in a signal-to-noise ratio of 4.

2.6. Reproducibility and accuracy

Both within- and between-day reproducibilities were determined. Two concentrations of drug (125 and 500 ng/ml) were included in these studies. For within-day reproducibility, six replicates of each sample were tested on the same day and the resulting percent relative deviation (reproducibility) and percent relative error (accuracy) determined. To measure between-day reproducibility, two concentrations of drug (125 and 500 ng/ml) were run in triplicate on each of three separate days. Percent relative deviation and percent errors were determined.

2.7. Dihydrocostatolide pharmacokinetics

Preliminary pharmacokinetics of DC were determined in C3H male mice obtained from the NCI. Drug was prepared for injection by dissolv-

ing it initially in dimethylsulfoxide then adding aliquots of Tween 20, Tween 80 and RPMI-1640 tissue culture medium (Whittaker Bioproducts, Walkersville, MD, USA) containing 15% fetal calf serum (2:1:1:16, v/v). Mice were administered DC at a dose of 20 mg/kg by either an intraperitoneal or intravenous route. Samples of blood were obtained from groups of 3 mice each at 2, 5, 15, 30 min, 1 h, 2 and 4 h after injection of drug. Plasma was obtained from the blood samples and frozen at −20°C until analysis.

3. Results and discussion

This is the first report of an analytical method for determining DC concentrations in biological

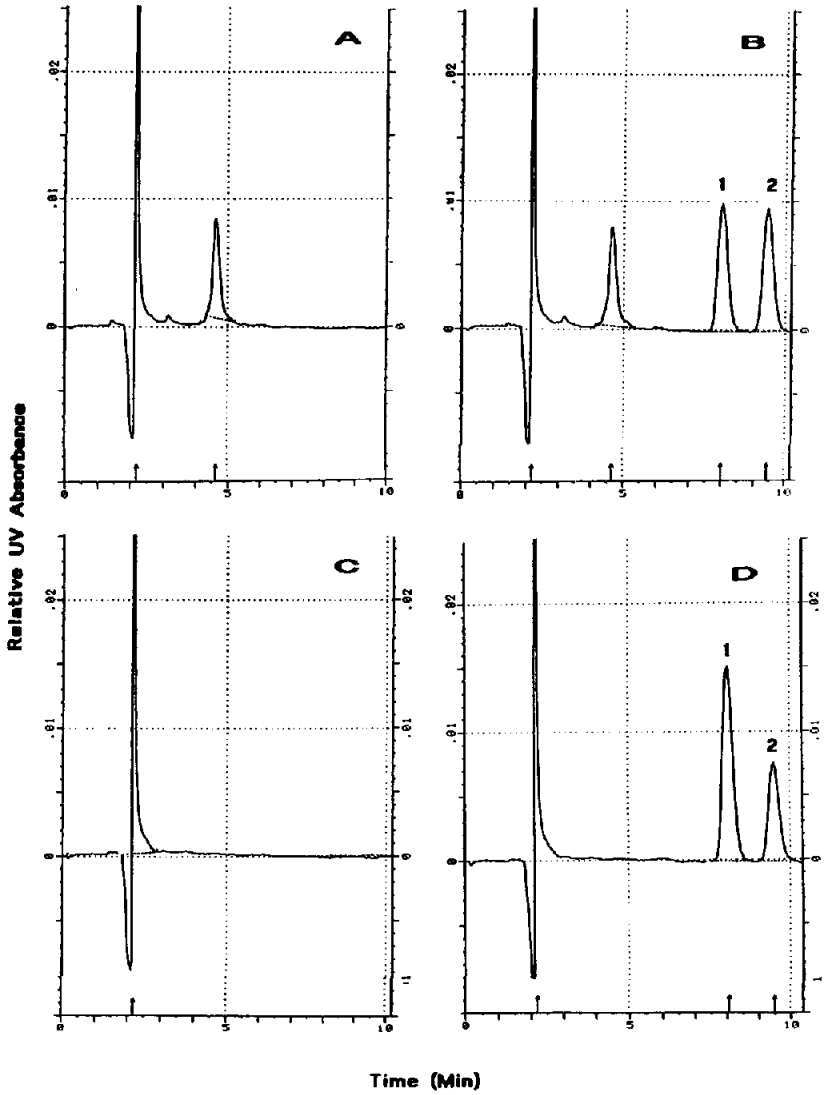

Fig. 2. HPLC chromatograms of (A) human blank plasma, (B) human plasma containing the internal standard, C (peak 1: 250 ng/ml) and DC (peak 2: 625 ng/ml), (C) mouse blank plasma, and (D) mouse plasma containing C (peak 1: 250 ng/ml) and DC (peak 2: 312 ng/ml). Detector sensitivity, 0.001 AUFS.

matrices such as plasma. The assay has been found suitable for measuring drug concentrations likely to be encountered in future anti-HIV clinical trials of this compound. Although the data are not presented, the assay was also found to be suitable for determining DC levels in urine as well with only minor modifications of the solid-phase extraction procedure. Costatolide, the internal standard used in the present assay, may also be developed as a potential anti-HIV agent. In that case, replacing DC with C in the present assay would provide a method suitable for quantitation of C as well.

Shown in Fig. 2 are typical chromatograms obtained from analyses of DC in mouse and human plasma. Chromatograms of extracted blank plasmas and of plasmas containing DC, with C as the internal standard, are presented. As can be seen, both DC and C are resolved from endogenous peaks. Retention times in mouse plasma were 8.1 min for C and 9.5 min for DC; in human plasma they were 8.0 min for C and 9.5 min for DC. The peak appearing at approximately 4.7 min in the human plasma is due to an unknown contaminant; however, its appearance in the C- and DC-spiked plasma did not interfere with quantitation of DC.

3.1. Assay reproducibility and accuracy

Within-day and between-day reproducibilities for human and murine plasma DC concentrations are shown in Tables 1 and 2, respectively. In Table 1 the relative standard deviations were less than 2% and 3% for human and mouse plasma samples, respectively. Percent relative errors ranged from 4.4 to 12.0% in human plasma and from 13.8 to 15.2% in mouse plasma. With between-day studies (Table 2), the relative standard deviations were low, ranging from 1.2 to 3.6 for human plasma and from 0.9 to 4.1 for murine plasma. Accuracies (as percent relative errors) were also excellent, ranging from 0.8 to 11.2 and from 1.6 to 8.2 for human and murine plasma, respectively.

3.2. Linearity

For both human and mouse plasma, the limit of detection of DC was 15 ng/ml. The linearity study was carried out over 3 days with concentrations ranging from 19.5 to 625 ng/ml in both human and mouse plasma. The coefficients of correlation between the DC/C peak-area ratio and DC concentrations in human and mouse plasmas were 0.999 for both plasma matrices. The equations resulting from the analysis of the regression plots for DC in human and murine plasma were $y = -0.0014 + 0.0017x$ and $y = -0.0049 + 0.0015x$, respectively.

3.3. Extraction recoveries

The extraction recoveries ($n = 5$) from plasmas spiked with DC (125 and 500 ng/ml) were $87 \pm 0.02\%$ and $104 \pm 0.03\%$ for human plasma and

Table 1
Within-day reproducibility and accuracy: dihydrocostatolide assay

Nominal conc. (ng/ml)	Calculated conc. mean ± S.D. (ng/ml)	Percent relative deviation[a]	Percent relative error[b]
Human plasma			
125	140 ± 2	1.77	12.0
500	522 ± 9	1.81	4.4
Mouse plasma			
125	144 ± 3	2.18	15.2
500	569 ± 10	1.78	13.8

[a] % Relative deviation: [standard deviation (calculated mean)/calculated mean] · 100.
[b] % Relative error: [(calculated mean − nominal value)/nominal value] · 100.

Table 2
Between-day reproducibility and accuracy: dihydrocostatolide assay

Day	Normal conc. (ng/ml)	Calculated conc. mean ± S.D. (ng/ml)	Percent relative deviation (%R.S.D.)	Percent relative error
Human plasma				
1	500	520 ± 7	1.4	4.0
2	500	496 ± 11	2.2	0.8
3	500	502 ± 16	3.1	0.4
1	125	139 ± 2	1.5	11.2
2	125	120 ± 4	3.6	4.0
3	125	131 ± 2	1.2	8.8
Mouse plasma				
1	500	541 ± 5	0.9	8.2
2	500	519 ± 9	1.7	3.8
3	500	537 ± 8	1.5	7.4
1	125	135 ± 4	3.0	8.0
2	125	131 ± 2	1.5	4.8
3	125	123 ± 5	4.1	1.6

84 ± 0.01% and 100 ± 0.05% for mouse plasma, respectively. The variance in recoveries observed for different concentrations of DC were controlled for by use of costatolide as the internal standard.

3.4. Pharmacokinetics

Drug concentration–time curves for DC after both i.p. and i.v. injection are presented in Fig. 3. The initial estimates of plasma DC half-lives derived from these pharmacokinetic data are 0.4 h and 3.1 h following i.v. or i.p. injections, respectively. More accurate assessments of DC half-lives, however, will depend on developing a more sensitive assay and using it to examine further time points out to at least 24 h.

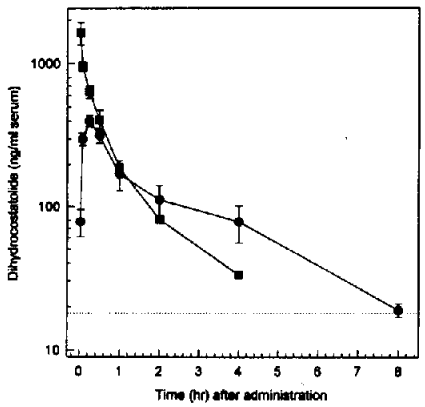

Fig. 3. Plot of DC plasma concentrations *versus* time obtained with mice administered drug (20 mg/kg) by either intravenous (–■–) or intraperitoneal (–●–) routes. Data are presented as the mean ± S.D. of 3 determinations in separate mice per time point. The dashed line indicates the lower limit of detection.

The clearance of DC was 757 ml/h and the relative volume of distribution was 452 ml. After i.p. injection, the T_{max} was 0.25 h and the C_{max} was 408 ng/ml. No DC metabolites were noted in either plasma or urine (data not shown).

Acknowledgement

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