Journal of Chromatography, 532 (1990) 442–444
Biomedical Applications
Elsevier Science Publishers B V , Amsterdam

CHROMBIO. 5451

Letter to the Editor

High-performance liquid chromatographic method for the analysis of 2′,3′-dideoxycytidine in human plasma

Sir,

Dideoxycytidine (ddC), a nucleoside analogue that potently inhibits the infectivity and cytopathic effects of HIV [1], is currently undergoing clinical testing in both adults and children with AIDS. In a recent phase I trial of ddC in children the doses utilized (0.015–0.040 mg/kg) [2] produced low concentrations of ddC in blood making it difficult to quantitate using existing methods [3].

In an effort to improve the ability to measure low concentrations of ddC, a paired-ion high-performance liquid chromatographic (HPLC) method used to measure the related dideoxynucleoside, dideoxyinosine (ddI) [4], was modified. This assay is capable of quantitating concentrations of ddC in human plasma as low as 0.03 μM using solid-phase extraction to isolate and concentrate the drug and paired-ion chromatography on a C_{18} column to elute the extracted sample. This report describes the details of this assay and presents the results of a pharmacokinetic study in a child treated with 0.04 mg/kg ddC both orally and intravenously.

EXPERIMENTAL

Chemicals and equipment

2′,3′-Dideoxycytidine was provided by the Pharmaceutical Resources Branch, National Cancer Institute (Bethesda, MD, U.S.A.). 5-Methyldeoxycytidine (5-MedC), internal standard, was purchased from Sigma (St. Louis, MO, U.S.A.). Ultrafree-MC filter units were purchased from Millipore (Bedford, MA, U.S.A.). The sources for other materials, reagents, and equipment were previously described [1].

Plasma preparation

Precautions used when handling specimens from HIV-infected individuals were previously described [1,5–8]. Solid-phase extraction was employed to clean-up and concentrate plasma samples as follows. Sep-Pak C_{18} cartridges were prewashed with 6 ml of methanol followed by 12 ml of water, loaded with 1.25 ml of plasma (0.5 ml/min), washed with 1.5 ml of water and eluted with 2 ml of methanol (0.5 ml/min). After evaporating to dryness under a stream of nitrogen, sam-

ples were taken up in 200 μl of water and filtered through Ultrafree-MC 0.45-μm filter units spun in an HBI microcentrifuge at 11 900 *g* for 10 min.

HPLC method

Extracted sample (100 μl) was injected onto the C_{18} column and eluted with 0.1% heptafluorobutyric acid with 6.5% acetonitrile at a flow-rate of 2.0 ml/min. The effluent was monitored for ddC at 288 nm and for the internal standard, 5-MedC, at 306 nm using a variable-wavelength UV detector.

RESULTS AND DISCUSSION

Elution times were 3.8 and 5.8 min for 5-MedC and ddC, respectively. Recovery of ddC and 5-MedC over a concentration range of 0.04–0.60 μ*M* for ddC and at 5 μg/ml for 5-MedC was 87±9 and 77±4%, respectively. Under the conditions described, the lower limit of detection for ddC was 0.03 μ*M*. This allows for a signal-to-noise ratio of 3.0. Standard curves, done in triplicate, were processed over a concentration range of 0 04–0.60 μ*M* using spiked normal plasma samples. Linear regression analysis of the peak-height ratios of ddC to internal standard *versus* the concentration of ddC gave a correlation coefficient of 0.97, using a reciprocally weighted linear regression analysis (forced through the origin) [9].

Fig. 1 shows the ddC plasma concentration *versus* time profile in a fourteen-year-old boy with AIDS who was monitored with frequent plasma samples following 0.040 mg/kg ddC administered as a 1-h intravenous infusion on the first day of therapy and followed by the same dose administered orally on the second day. Peak plasma ddC concentrations for the intravenous and oral doses were 0.28 and 0.19 μ*M*, respectively.

The low plasma concentrations of ddC which resulted from the low dose range

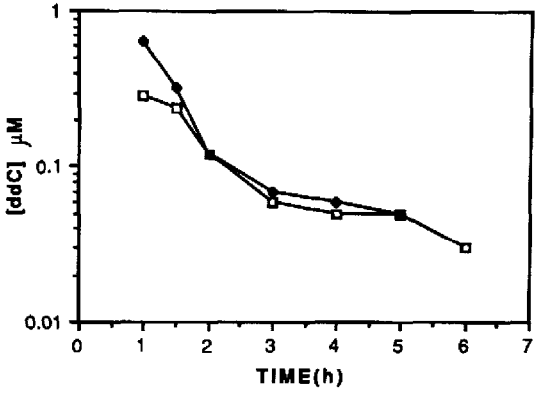

Fig 1 Plasma concentration *versus* time profile of ddC in a fourteen-year-old patient after a 0 04 mg/m^2 intravenous dose (□) and after the same dose administered orally (■). Samples were drawn at time intervals indicated

administered presented a challenge since the previously used method allowed measurement of only the peak levels. Because ddC binds to this column so tightly under these conditions, most of the endogenous interferences are washed off the column before the ddC peaks appears. It is because of this that a greater quantity of plasma can be prepared and more sample can be injected thereby resulting in greater sensitivity. As evidenced by the patient data presented, this has made it possible to assess pharmacokinetics of this drug in children.

This assay technique also may be applicable to analysis of other purine and pyrimidine analogues. Preliminary results from examining other cytidine analogues are promising.

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(First received March 12th 1990; revised manuscript received June 5th, 1990)