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A rapid, simple and accurate method for the bioanalysis of zidovudine

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Summary

A rapid and simple method has been developed for the bioanalysis of zidovudine, a new antiviral drug, which has proven to show some effectiveness in the treatment of AIDS and AIDS-related complex. After extraction of the compound from the biological matrix with ethyl acetate, evaporation of the organic solvent and reconstitution of the residue with methanol reversed-phase HPLC on RP8 material is applied with 1% acetate buffer pH 5–methanol (82/18, v/v) as the solvent, and detection at 265 nm. Detection limits in plasma and urine were 20 and 200 ng/ml, respectively. The method has been validated with a single dose pharmacokinetic study in an AIDS patient.

Introduction

Zidovudine, also known as azidothymidine (AZT) and Retrovir, is an antiviral drug which has attracted a lot of attention after the recent discovery of a certain effectiveness in the symptomatic treatment of acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. Being a thymidine analogue (Fig. 1) it shows in vitro antiviral activity against retroviruses, including the AIDS-related human immunodeficiency virus (HIV) (Mitsuya et al., 1985; Furman et al., 1986) and recently the in vivo virostatic effect against HIV has been confirmed (Yarchoan et al., 1986). Since then a large number of studies has been published on the pharmacological activity of the compound; however, physicochemical and analytical information on the drug is scarce.

Zidovudine is administered in fairly high doses (2.5–3.5k mg/kg b. wt.) both orally and i.v., in continuous dosage regimens (Klecker et al., 1987).

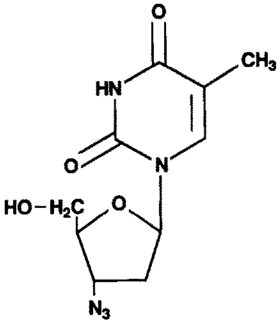

Fig. 1. Structure of zidovudine.

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Although some information on the pharmacokinetics of zidovudine is available (Klecker et al., 1987), the analytical methodology used in that study is rather time-consuming and seems not very suitable for routine analysis in pharmacokinetic studies and drug monitoring. In this study a simple and rapid method for the bioanalytical determination of zidovudine is developed, based on reversed-phase HPLC with UV detection after extraction of the drug from plasma or urine. The method has been validated in a single dose pharmacokinetic study in an AIDS patient.

Materials and Methods

Chemicals

Zidovudine was isolated by extraction with methanol from a Retrovir capsule, containing 100 mg zidovudine, obtained from the Slotervaart Hospital, Department of Pharmacy in Amsterdam. After filtration the methanol extract was transferred to a volumetric flask of 100 ml and brought to volume with methanol. This solution, containing 1 mg/ml zidovudine, was used as the stock solution. This procedure had to be adopted since no pure zidovudine substance was made available to us by the manufacturer.

All other chemicals were of analytical grade and deionized water was used throughout.

Apparatus

UV-visible absorption spectra were recorded on a Perkin Elmer Lambda 5 UV/VIS Spectrophotometer (Perkin Elmer, Oak Brook, IL, U.S.A.).

High-performance liquid chromatographic (HPLC) analysis was performed using a Model 510 solvent delivery system, a U6K injector (both from Waters Ass., Milford MA, USA) and a Spectroflow 773 Absorbance Detector (Kratos, Analytical, Ramsey, NJ, U.S.A.).

The analytical column (300 × 3.9 mm) was filled with Lichrosorb RP8 (10 μm) material, while prior to the analytical column a guard column (20 × 4 mm) was placed, slurry-packed with Lichroprep RP8 (5–20 μm). The solvent consisted of 1% acetate buffer pH 5–methanol (82/18, v/v) with a flow of 1.5 ml/min.

UV detection was carried out at the wavelength of maximum absorbance, viz. 265 nm.

Analytical procedure

1.0 ml plasma or urine, containing zidovudine, is vortex-mixed with 2.0 ml ethyl acetate for 30 s. The layers are separated by centrifugation for 5 min at 4000 rpm. 1.0 ml of the organic layer is evaporated at 50 °C under a stream of nitrogen and the residue redissolved in 100 μl methanol by vortex-mixing for 5 s. Of this solution 20 μl is subsequently injected into the chromatographic system and the amount of zidovudine is determined by measuring the height of the zidovudine peak.

Pharmacokinetic procedure

A pharmacokinetic study was performed in an AIDS patient after receiving orally a single dose of 300 mg zidovudine (5 mg/kg b. wt.) Blood samples were collected at 15, 30, 45, 60 and 90 min after the dose and subsequently 2, 4, 6 and 8 h after the dose.

Urine samples were obtained 3, 5, 7 and 7.5 h after the administration of the drug. Blood samples were collected in tubes containing heparin solution and, after removal of the blood cells by centrifugation, 1.0 ml plasma was subjected to the analysis. Urine samples did not need any pretreatment prior to the analytical procedure.

Results and Discussion

UV-VIS spectroscopy

The UV-VIS absorbance spectrum of zidovudine revealed only one maximum at 265 nm with a UV absorbance sufficiently high for HPLC detection. All chromatographic experiments were therefore executed with UV detection at that wavelength.

Clean-up procedure

The isolation of zidovudine, being a fairly polar compound, from aqueous solutions and biological fluids can be expected to be tedious. A number of extraction procedures have been tested. No success was obtained with solid phase extraction of the compound using various sorbents, such as silica and silica coated with octadecyl, cyano and dihydroxy groups. The elution of the compound from these phases, even with water, was too fast to enable the removal of possibly interfering endogenous material. Extraction from aqueous solutions with various organic solvents revealed that only ethyl acetate was suitable since with this extraction fluid a sufficiently high recovery of about 85% could be obtained. In preliminary experiments with plasma and urine samples spiked with various amounts of zidovudine, recoveries in the same order of magnitude were obtained. The application of an internal standard was, for this reason, considered to be unnecessary.

Chromatography

For the HPLC analysis of zidovudine a number of sorbents and solvents have been tested. From these tests it became obvious that a reversed-phase system with RP8 material as the sorbent and a solvent containing a low concentration of organic modifier would give the best results. Finally, a Lichrosorb RP8 (10 μm) column with a guard column also containing RP8 material to diminish contamination of the analytical column, and a mixture of acetate buffer pH 5 and methanol (82/18, v/v) turned out to be the optimal system for the analysis of zidovudine in biological fluids, provided that the zidovudine-containing ethyl acetate extract was evaporated and redissolved in methanol, in order to avoid chromatographic disturbances caused by the ethyl acetate and to obtain reproducible results. Moreover, this step also served as a concentration of the sample, thus increasing the sensitivity of the assay. Fig. 2 represents a typical chromatogram of a plasma sample, spiked with zidovudine, while in Fig. 3 the chromatography of a similar urine sample is shown. The sensitivity of the method is satisfactory: with an absolute detection limit of 2 ng (signal-to-noise ratio 3) in water the limits for detection in plasma and urine are 20 ng/ml and 200 ng/ml, respectively. The reproducibility and precision of the assay are also sufficient: calibration curves in plasma in the ranges 20–100 ng/ml, 100–1000 ng/ml and 10–100 μg/ml show good linearity ($r > 0.999$). The same applies for calibration curves

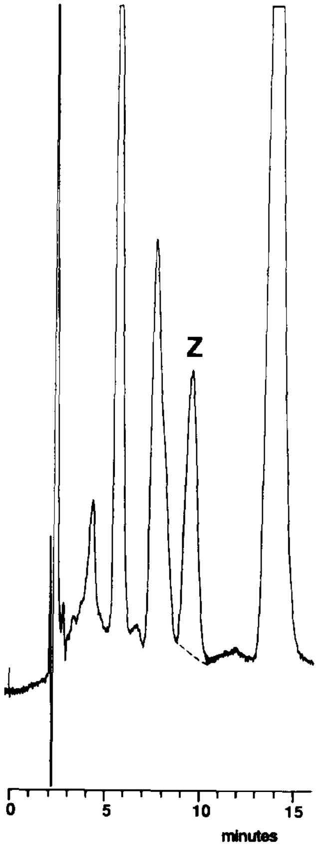

Fig. 2. HPLC chromatogram of a plasma sample spiked with zidovudine (Z) (600 ng/ml).

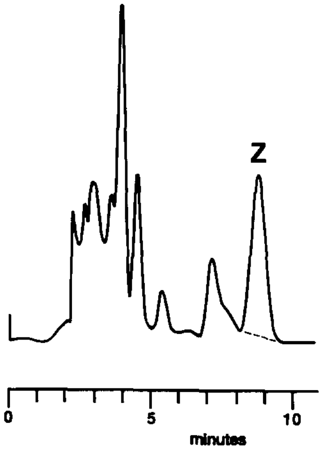

Fig. 3. HPLC chromatogram of a urine sample spiked with zidovudine (Z) (10 μg/ml). The broken line presents the blank chromatogram. For chromatographic conditions see text.

in urine in the ranges 200–1000 ng/ml and 5–1000 μg/ml. However, the urinary calibration curve in the lower concentration range deviates slightly from the origin, indicating a small endogenous peak co-eluting with the zidovudine peak. This co-elution occurs with all solvents tested. Recoveries and standard deviations of the plasma assay vary from 83 ± 8% in the lower concentration range (60 ng/ml) to 85 ± 2.5% in the upper region (60 μg/ml). For the urine assay these values vary from 84 ± 9% (300 ng/ml) to 86 ± 2.5% (60 μg/ml).

Pharmacokinetics

The analytical method described was validated with a single-dose pharmacokinetic study in an AIDS patient receiving orally a dose of 300 mg zidovudine (5 mg/kg b. wt.). Fig. 4 shows the concentration–time profile of zidovudine in the patients' plasma, while Table 1 gives an overview of the urinary concentrations. Unfortunately, due to accidental loss of the sample prior to determination, the 3 h urine sample could not be analyzed.

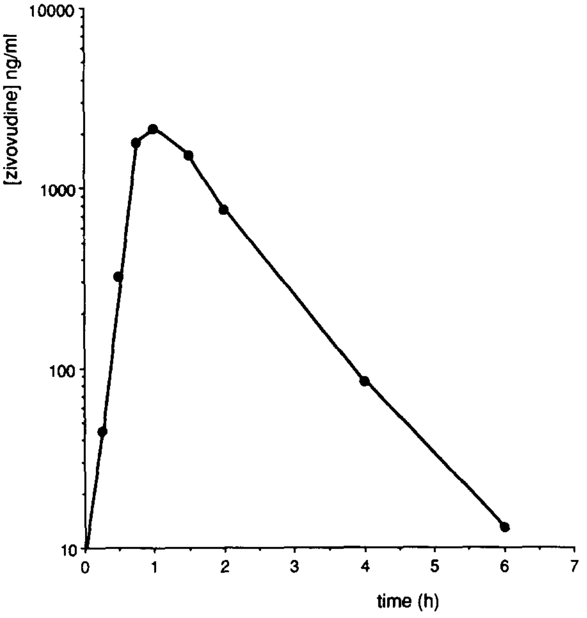

Fig. 4. Plasma concentration-time profile of zidovudine after oral administration of a single dose (300 mg) to an AIDS patient.

TABLE 1

Urinary excretion of zidovudine

Sample time (h)	Volume (ml)	Zidovudine (μg/ml)	Total zidovudine (mg)
3	100	n.d.	n.d.
4.8	500	19.6	9.8
6.8	200	16.2	3.2
7.3	100	4.8	0.5

n.d., not determined.

Fig. 4 shows a rapid uptake of zidovudine from the gastrointestinal tract with a maximum plasma concentration of 2.1 μg/ml, reached after 60 min, and a first order elimination of the drug with a half-life of 0.7 h. Table 1 shows only a small fraction of the drug eliminated unchanged in the urine. Considering the fact that no data concerning the 3 h urine sample are available, this pattern is well in accordance with literature data. The same applies to the shape of the plasma concentration–time profile, the maximum plasma concentration and the half-life for the elimination of the drug (Klecker et al., 1987). These observations are proof for the validity of the bioanalytical zidovudine assay for routine purposes.

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