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Specific radioimmunoassays for the measurement of stavudine in human plasma and urine

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Abstract

Sensitive and specific radioimmunoassays (RIAs) have been developed and validated for the determination of stavudine, a nucleoside analog possessing anti-human immunodeficiency virus (HIV) activity, in human plasma and urine. The hemisuccinate of stavudine was conjugated with histamine and radioiodinated to yield the radiotracer. Antisera were produced by injecting the immunogen, stavudine-hemisuccinate-bovine thyroglobulin, into rabbits. The antisera exhibited high specificity for stavudine as the structurally related analogs and other anti-HIV agents did not interfere in the assays. The methods could reliably quantitate stavudine in plasma from 2.5–100 ng ml^{-1} and in urine from 5.0–1000 ng ml^{-1} (after 2.5-fold dilution) with good accuracy and precision. The lower limits of quantitation were 2.5 ng ml^{-1} in human plasma and 5.0 ng ml^{-1} in urine (after 2.5-fold dilution). The RIA methods were applied to the analysis of stavudine in plasma and urine obtained from HIV-infected patients receiving the drug in clinical trials.

Keywords: Antiretroviral agent; Clinical trials; Drug analysis; Pharmacokinetic studies; Radioimmunoassay; Stavudine

1. Introduction

Stavudine, 2′,3′-didehydro-3′-deoxythymidine, d4T, (Zerit®, Bristol-Myers Squibb, Princeton, NJ) is a new nucleoside analog that has been approved for the treatment of adults with advanced human immunodeficiency virus (HIV) infection [1]. In its clinical development, a dose as low as 0.033–0.67 mg kg^{-1} given three times daily (0.1–2.0 mg kg^{-1} day^{-1}) was evaluated in HIV-infected adults [2]. The safety, efficacy, and pharmacokinetics of stavudine were also evaluated in pediatric patients with HIV infection [3]. In these trials, low stavudine concentrations and limited biological sample volumes for assay were anticipated. The HPLC/UV assay methods for

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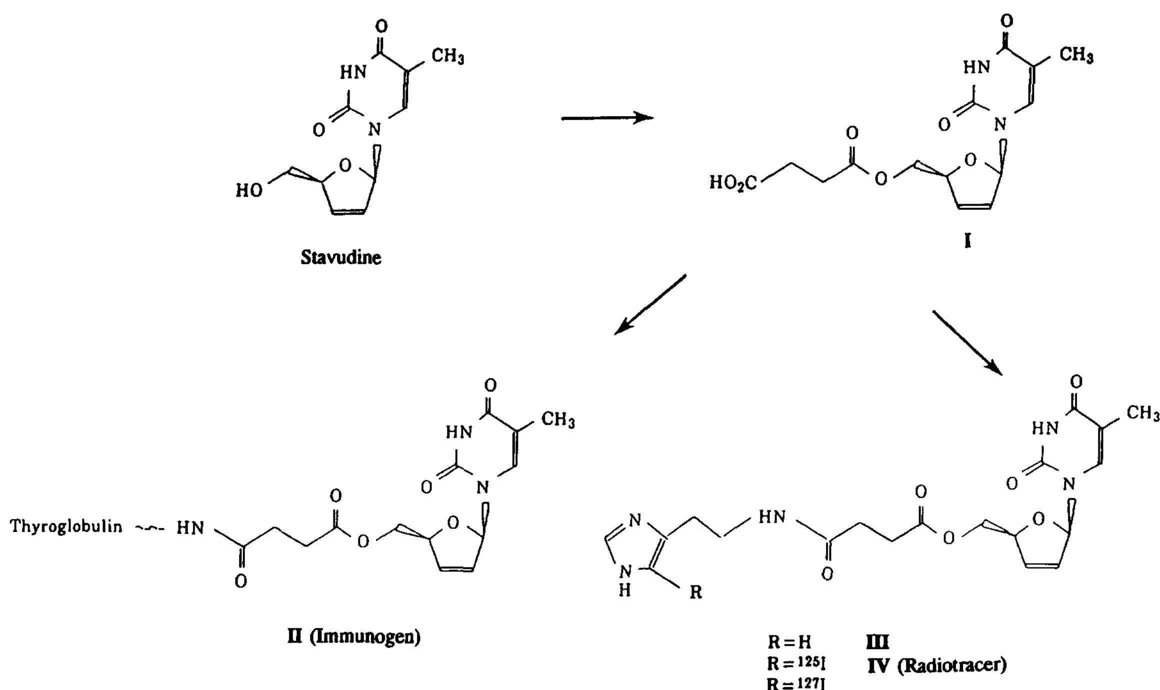

Fig. 1. Scheme for the synthesis of the immunogen and the radioligand in the stavudine RIA.

stavudine [4], which required a 0.5 ml sample, had a lower limit of quantitation (LLQ) of 25 ng ml^{-1} in plasma and 100 ng ml^{-1} in urine and were, therefore, considered inadequate for providing meaningful pharmacokinetic profiles at low doses in adult and pediatric patients. Consequently, development of sensitive, specific, and reproducible radioimmunoassays (RIAs) for stavudine in human plasma and urine was pursued in order to assess the pharmacokinetics of stavudine in adults and children with HIV infection.

2. Experimental

2.1. Materials

Stavudine was a product of Bristol-Myers Squibb Pharmaceutical Research Institute (Princeton, NJ). All other compounds used in this study were readily obtained from commercial sources.

2.2. Preparation of the immunogen

Stavudine (224 mg, 1 mmol), succinic anhydride (400 mg, 4 mmol), and triethylamine (270 μl, 2 mmol) were allowed to react in 10 ml of dry dimethylformamide, DMF, for 2 h at 60°C under nitrogen. The solvent was evaporated under reduced pressure. The residue was dissolved in the mobile phase consisting of chloroform–methanol–acetic acid in water (50:10:0.25, v/v/v) and subjected to chromatography on silica gel to yield 255 mg (79% yield) of **I** (Fig. 1) as a white solid. A 10 mg (0.031 mmol) sample of **I** was dissolved in 0.25 ml of dry DMF and cooled on ice. *N*-hydroxysuccinimide (12 mg, 0.11 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (20 mg, 0.11 mmol) were added and stirred for 3 h under nitrogen. The resulting solution was added dropwise to an ice-cold solution of thyroglobulin (20 mg, 0.3 μmol) dissolved in 10 ml of 0.1 M sodium bicarbonate buffer, pH 8.3. The solution was stirred overnight, then dialyzed extensively over 36 h against three changes of buffer (50 mM phosphate buffer, pH 7.4, containing 150

mM NaCl) to yield the immunogen (**II**, Fig. 1). The extent of conjugation of stavudine to thyroglobulin was estimated from UV spectral studies to be 50:1.

2.3. Preparation of the radiotracer

N-hydroxysuccinimide (40 mg, 0.35 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (67 mg, 0.35 mmol) were added to an ice-cold solution of **I** (50 mg, 0.15 mmol, in 1 ml of dry DMF) and the reaction mixture stirred under nitrogen for 3 h. After this time, a solution of histamine (39 mg, 0.35 mmol) and triethylamine (20 μl) dissolved in 0.5 ml of dry DMF was added. After overnight stirring at room temperature, the solvent was evaporated under reduced pressure. The residue was subjected to chromatography on silica gel, eluting with a stepwise gradient of chloroform–methanol–acetic acid in water, starting at 40:10:0.5, v/v/v, and ending at 30:10:0.5, v/v/v. The resulting white solid was dissolved in acetone, filtered to remove any insoluble material (silica gel), and the filtrate evaporated under reduced pressure to afford 40 mg (62% yield) of **III** (Fig. 1) as a white solid. A solution of **III** (50 μg, 0.15 mmol) in 0.2 M citrate buffer (10 μl, pH 5.0) was treated at ambient temperature with $Na^{125}I$ (5 μl, 5 mCi). The radioiodination was initiated by the addition of a citrate buffer solution (0.25 M, pH 5.0) of chloramine-T (20 μl, 0.14 μmol). After 30 s, the reaction was quenched by addition of a citrated buffer solution (0.2 M, pH 5.0) of sodium metabisulfite (20 μl, 0.32 μmol). The reaction solution was diluted with 100 μl of mobile phase and the radiotracer (**IV**, Fig. 1) was isolated by HPLC (Zorbax Rx C8, 4.6 mm × 250 mm; 50 mM potassium phosphate pH 7.4–methanol, 68:32; 1 ml min^{-1}; radiotelemetric detection). The radiochemical yield was 53%.

2.4. Production of antisera

New Zealand white rabbits were purchased by, and housed at, the Laboratory Animal Center, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ. The rabbits were initially injected by the subcutaneous route with the immunogen solution that was emulsified with an equal volume of Freund's complete adjuvant in doses ranging from 50–200 μg per injection. Two to three booster injections of the same doses with an equal volume of Freund's incomplete adjuvent were given every 4 weeks. The rabbits were bled in 10–14 day intervals after each booster injection. The antisera were selected on the basis of maximum binding, titer, sensitivity, and specificity.

2.5. Preparation of the separant

The separant for the plasma RIA was prepared immediately before use by adding 1 ml of 0.45 μm membrane-filtered goat anti-rabbit gamma globulin (GARG) to 80 ml of polyethylene glycol 8000 (PEG) and 0.1% sodium azide. Pansorbin® cell suspension was used as a separant in the urine RIA.

2.6. Preparation of assay solutions

Stavudine stock solution (1 mg ml^{-1}), stavudine calibration standards (2.5–1000 and 5–1000 ng ml^{-1} for plasma and urine assays respectively) and rabbit antisera (1.25% v/v) were prepared in, or diluted with, 0.05 M potassium phosphate buffer, pH 6.8, containing EDTA (1 mg ml^{-1}), sodium azide (1 mg ml^{-1}), sodium chloride (9 mg ml^{-1}), and bovine serum albumin (BSA) fraction V (2 mg ml^{-1}). The calibration standards were aliquoted into vials, capped, and stored at −70°C until use. The radiotracer was diluted (to 3 μCi ml^{-1}) with 0.2 M citrate–phosphate buffer, pH 5.2, containing EDTA (1 mg ml^{-1}), sodium azide (1 mg ml^{-1}), BSA (2 mg ml^{-1}), and 8-anilino-1-naphthalene sulfonic acid (0.16 mg ml^{-1}). The diluted radiotracer solution was stable for at least 6 weeks at 4°C.

2.7. Plasma RIA procedure

The experimental design consisted of two total count (TC) tubes, two non-specific binding (NSB) tubes, two zero-binding tubes (B_0), two tubes for each concentration of plasma standard (0, 2.5, 5, 10, 25, 50 and 100 ng ml^{-1}), three tubes for each

concentration of plasma quality control sample (5, 50 and 80 ng ml^{-1}), and one tube of test plasma sample. 10 μl portions of the appropriate samples were added to each of the tubes, followed by the addition of either 10 μl of blank plasma (to buffer standards) or 10 μl of buffer (to test and quality control samples). An additional 75 μl of blank plasma was added to all except the TC tubes. Then, 100 μl of antibody solution was added to all but the TC and NSB tubes. The tubes were covered with plastic wrap, vortexed for 30 s, and incubated overnight at 4°C. The next day, 200 μl of ^{125}I-histamine–stavudine conjugate was added. After incubation for 4 h at 4°C, 1 ml of cold freshly prepared PEG–GARG separant was added to all except the TC tubes. The tubes were vortexed for 30 s and incubated for 10 min at 4°C. The samples were centrifuged at 3000 rev min^{-1} for 15 min at 4°C to separate bound from free antigen. The liquid was drained for at least 10 min and the tubes containing the pellets were counted in a gamma counter for 5 min in the ^{125}I window.

2.8. Urine RIA procedure

The experimental design consisted of two TC tubes, two NSB tubes, two zero-binding tubes, two tubes for each concentration of urine standard (0, 5, 10, 25, 50, 100, 250, 500 and 1000 ng ml^{-1}), three tubes for each concentration of plasma quality control sample (75, 200 and 500 ng ml^{-1}), and one tube of urine test sample. Appropriate volumes of the samples (10 μl of test and quality control samples or 25 μl of the buffer standard) were added to each of the tubes followed by the addition of either 10 μl of blank urine (to buffer standards) or 25 μl of buffer (to test and quality control samples). Pansorbin® cell suspension (50 μl) and additional blank urine (90 μl to test and quality control samples and 100 μl to buffer standards) were added to all except the TC tubes. The mixture was incubated for 10 min at room temperature and then 200 μl of ^{125}I-histamine–stavudine conjugate to all tubes and 100 μl of antibody solution to all but the TC and NSB tubes were added. After incubation for 16 h at 4°C, 2.5 ml of buffer containing 1 mg ml^{-1} BSA was added to all except the TC tubes. The samples were centrifuged at 3200 rev min^{-1} for 15 min at 4°C to separate the bound from free antigen. The liquid was drained and the tubes containing the pellets were counted in a gamma counter for 2 min in the ^{125}I window.

2.9. Data analysis

A detailed description of the procedures and laboratory data management systems used to collect and process data for the study has been previously reported [5]. Standard curves were constructed using a four-parameter logistic model [6] as shown below:

$$\text{counts} = \text{NSB} + \frac{B_0 - \text{NSB}}{1 + \left(\frac{C}{\text{ED}_{50}}\right)^b}$$

where "counts" is the number of radioactive counts for 5 (plasma) or 2 (urine) min, C is the concentration, B_0 is an estimate of maximum binding, NSB is an estimate of nonspecific binding, ED_{50} is an estimate of concentration at 50% binding, and b is the slope coefficient. Residuals from the standard curve were calculated and printed for testing potential outliers. Outliers were evaluated using the test described by Dixon and Massey [7] at the $p = 0.01$ significance level. If an outlier was detected, the standard was flagged as "statistical rejection" and the curve was recalculated without the aberrant value.

3. Results

3.1. Standard curve characteristics

In order to assess the consistency of the relationship between the response and the concentration of stavudine, the ratios of percent bound to unbound ($\%B/B_0$) for the seven standards from three runs per matrix are listed in Table 1. The variations (measured as % relative standard deviation, RSD) were less than 4% and 7% for the plasma and urine assays respectively. The concentration–response curve was well described by the four-parameter logistic model (Fig. 2) in the two

Table 1
Binding statistics for standards in stavudine RIA in human plasma and urine[a]

Run #	% B/B_0 for standards at plasma concentrations (ng ml^{-1}) of							
	2.5	3	5	10	25	50	100	
1	80.0	73.3	69.1	54.4	42.1	33.0	25.8	
	79.3	75.2	68.6	56.1	40.5	32.1	25.2	
2	78.3	73.2	69.1	55.3	40.0	32.8	24.6	
	78.3	74.6	67.5	56.9	41.1	31.1	23.6	
3	77.7	76.7	68.5	56.0	39.9	32.3	23.8	
	78.5	76.3	68.6	55.3	40.9	32.0	23.7	
Mean	78.7	74.9	68.6	55.7	40.8	32.2	24.5	
SD	0.83	1.47	0.59	0.86	0.81	0.67	0.91	
RSD (%)	1.0	2.0	0.9	1.5	2.0	2.1	3.7	
Run #	% B/B_0 for standards at urine concentrations (ng ml^{-1}) of							
	5	10	25	50	100	250	500	1000
1	84.1	75.5	62.2	50.2	38.9	24.9	19.0	15.6
	84.4	76.6	61.5	49.4	38.3	26.6	19.6	15.3
2	84.2	76.5	63.4	52.1	40.1	28.1	22.4	17.4
	85.1	75.6	64.7	51.4	40.1	27.9	22.2	16.7
3	83.2	71.9	61.2	49.0	37.2	25.3	20.1	15.9
	83.1	73.5	60.2	48.5	36.5	24.3	20.1	15.9
Mean	84.0	74.9	62.2	50.1	38.5	26.2	20.6	16.1
SD	0.78	1.87	1.63	1.40	1.47	1.59	1.40	0.79
RSD (%)	0.9	2.5	2.6	2.8	3.8	6.1	6.8	4.9

[a] B = estimate of binding in the presence of a given concentration of stavudine standard; B_0 = estimate of maximum binding.

matrices with r^2 values exceeding 0.996. The standard curve parameters are summarized in Table 2. For the plasma assay, the variations were 5.1% RSD for the slope coefficient (b) and 17.2% RSD for the estimate of concentration at 50% binding (ED_{50}); the corresponding values for the urine assay were 0.6% RSD and 8.7% RSD for b and ED_{50} respectively.

3.2. Specificity

The specificity of the RIA was studied by examining the cross-reactivity of stavudine antibody with a variety of structurally related compounds. Cross-reactivity, expressed as the percentage ratio of stavudine ED_{50} to cross-reactant ED_{50}, is summarized in Table 3. None of the compounds showed any significant cross-reactivity to the antibody. In addition, no interference with the quantitation of stavudine was observed when blank plasma or urine specimens from different sources were used.

3.3. LLQ

To assess the LLQ of the assay methods, plasma samples from 10 different individuals were spiked with stavudine (2.5 and 5.0 ng ml^{-1}) and urine samples from six individuals were spiked with stavudine (12.5, 25 and 50 ng ml^{-1}). Taking

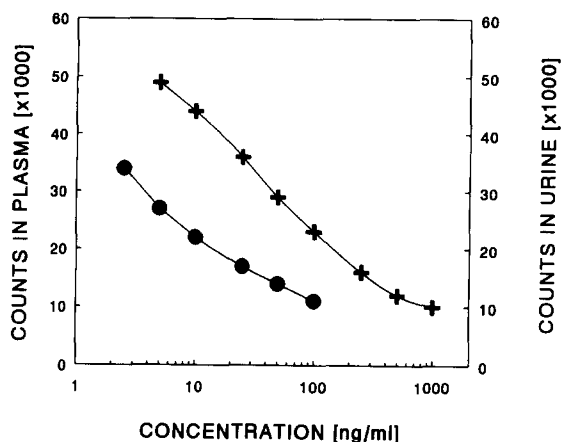

Fig. 2. Standard curves of the stavudine RIA in human plasma (●) and urine (+).

into account the dilution factor of 2.5 in the urine RIA, these concentrations corresponded to 5, 10 and 25 ng ml^{-1} respectively. The criteria for the selection of the LLQ were that precision, as determined by %RSD, and accuracy, as determined by % deviation from nominal values, be no greater than 20%. Based on the precision values (8% RSD for plasma and 13% RSD for urine) and the accuracy values ($\leq$ 2% deviation for both plasma and urine samples), the LLQ of the plasma and urine RIA methods was estimated at 2.5 and 5 ng ml^{-1} respectively.

Table 3
Cross-reactivity of potential metabolites, potential coadministered drugs, and endogenous nucleosides in stavudine RIA in human plasma and urine

Compound	% Cross-reactivity (Stavudine ED_{50}/ cross-reactant ED_{50}) × 100
Adenine	< 0.01
Adenosine	< 0.01
Dideoxyadenosine	< 0.01
Dideoxycytidine	< 0.01
Dideoxyguanosine	< 0.01
Dideoxyinosine	< 0.01
5-Fluorouracil[a]	< 0.01
5-Fluorocytosine[a]	< 0.01
Guanine[a]	< 0.01
Guanosine	< 0.01
α-Thymidine	< 0.01
Thymine[a]	< 0.01
Zidovudine	< 0.12

[a]Not tested for cross-reactivity in urine RIA.

3.4. Precision and accuracy

To assess the precision of the assay, quality control samples at three concentrations of stavudine in plasma (5.0, 50 and 80 ng ml^{-1}) and urine (75, 200 and 500 ng ml^{-1}), representing the entire range of the standard curve, were studied. The samples were assayed in six replicates in three

Table 2
Standard curve parameters for stavudine RIA in human plasma and urine

Matrix	Run #	NSB (counts)[a]	B_0 (counts)[a]	ED_{50} (ng ml^{-1})	b
Plasma	1	5729	42 729	7.43	0.704
	2	5347	39 300	10.5	0.752
	3	5271	40 415	9.31	0.779
	Mean	5449	40 815	9.09	0.745
	SD	245	1749	1.56	0.038
	RSD (%)	4.5	4.3	17.2	5.1
Urine	1	5744	57 202	39.8	0.772
	2	6027	52 321	42.9	0.773
	3	6467	56 352	36.0	0.780
	Mean	6079	55 292	39.6	0.775
	SD	364	2608	3.5	0.004
	RSD (%)	6.0	4.7	8.7	0.6

[a]Radioactive counts per 5 (plasma) or 2 (urine) min.

Table 4
Precision and accuracy of stavudine RIA in human plasma and urine

Matrix	Sample type	Concentration ng ml^{-1})		n	Precision (%RSD)	Accuracy (%Dev.)
		Nominal	Mean observed (SD)			
Plasma	Unblinded	5.00	5.08 (0.36)	18	7.1	1.6
		50.0	50.1 (3.29)	18	6.6	0.3
		80.0	83.2 (4.91)	18	4.9	4.0
	Blinded	4.82	4.75 (0.11)	4	2.2	−1.6
		9.64	9.53 (0.19)	4	2.0	−1.1
		24.1	23.4 (0.56)	4	2.4	−2.8
		48.2	45.8 (0.60)	4	1.3	−5.0
		77.1	74.6 (1.87)	4	2.5	−3.2
		80.0	80.9 (1.13)	4	1.4	1.2
		482	468 (3.27)	4	0.7	−2.9
Urine	Unblinded	75.0	78.0 (5.47)	18	7.0	4.0
		200	203 (10.1)	18	5.0	1.3
		500	487 (23.8)	18	4.9	−2.6
	Blinded	15.0	14.9 (0.04)	4	0.3	−0.3
		51.6	53.0 (0.90)	4	1.7	2.8
		150	158 (0.63)	4	0.4	5.4
		310	322 (7.73)	4	2.4	4.0
		413	408 (3.26)	4	0.8	−1.3
		5162[a]	5052 (55.6)	4	1.1	−2.1

[a]Assayed after 20-fold dilution.

analytical runs. As shown in Table 4, there were no inter-run differences in the mean concentrations in the two matrices with both precision (% RSD) and accuracy (% deviation) values being under 8%. To assess assay accuracy, 28 blinded-plasma and urine samples spiked with stavudine were analyzed in a single run. There were four replicates of each of the seven concentrations. Table 4 shows that the precision and accuracy values at each concentration were less than 3% RSD and less than 6% deviation from the nominal value respectively.

3.5. Specimen stability

To evaluate the stability of stavudine in the plasma samples, three quality control samples at each of the three concentrations (5, 50 and 80 ng ml^{-1}) were assayed after storage at room temperature, at 4°C, and at −20°C. The results of this comparison are presented in Table 5. Stavudine was stable for at least 4 days at room temperature and at 4°C and for at least 1 year at −20°C. In addition, plasma and urine samples were found to be stable when subjected to two and three freeze–thaw cycles respectively.

3.6. Cross comparison of RIA and HPLC analytical procedures

Plasma and urine samples from three patients participating in a stavudine clinical trial were analyzed for stavudine by both the current RIA and a previously published HPLC procedure [4]. As shown in Figs. 3 and 4, the remarkable overlap of the mean plasma stavudine concentration–time profiles and urinary recovery in the three patients following three times daily oral administration of 0.67 mg kg^{-1} indicates a strong concurrence between the data generated by the two assay methods.

Table 5
Stability of stavudine in human plasma

Temperature (duration)	Concentration (ng ml^{-1})		n	Precision (%RSD)	Accuracy (% Dev.)
	Nominal	Mean observed (SD)			
Room temperature (4 days)	5.0	4.95 (0.02)	3	0.4	−1.6
	50.0	56.6 (2.32)	3	4.1	13.2
	80.0	84.4 (3.54)	3	4.2	5.4
4°C (4 days)	5.0	4.94 (0.05)	3	1.0	−1.6
	50.0	53.5 (0.43)	3	0.8	7.0
	80.0	83.9 (1.59)	3	1.9	4.8
−20°C (4 days)	5.0	4.54 (0.02)	3	0.5	−9.4
	50.0	56.5 (1.52)	3	2.7	13.0
	80.0	85.1 (0.34)	3	0.4	6.4
−20°C (365 days)	9.7	8.95 (0.68)	27	7.6	−7.7
	97.0	90.7 (7.40)	27	8.2	−6.5
	19 410[a]	17 361 (1299)	27	7.5	−10.6

[a]Assayed after 1000-fold dilution.

4. Discussion

Stavudine is a potent anti-HIV agent [8] which has been approved for the treatment of HIV infection [1]. The RIA methods described in this paper are a successful approach to monitoring plasma and urine levels of stavudine in clinical pharmacokinetic studies, especially following low doses. In addition, the plasma analysis is performed directly on the sample without prior extraction, allowing the use of a small sample volume (10 μl). The hemisuccinate of stavudine was readily linked to thyroglobulin to yield an immunogen that elicited the production of highly specific and sensitive antibody with very high titre in rabbits. The reaction yield of the immunogen was low (2%) due to the fact that stavudine hemisuccinate (I), used in the overnight conjugation reaction at pH 8.3, was probably unstable at high pH. The antibody showed no cross-reactivity to endogenous nucleosides. Coadministration of stavudine to patients concurrently receiving other anti-HIV agents was anticipated in early clinical trials. Dideoxyadenosine, dideoxcytidine, dideoxyinosine, and zidovudine had no significant effect on assay performance. Specifically, zidovudine and its glucuronide metabolite did not interfere with the assays at concentrations as high as 2.5 and 10 μg ml^{-1} respectively. Thus, the assays are specific for stavudine.

While both the plasma and urine RIA methods utilized the same basic reagents (antibody and radiolabel), due to matrix effects, there were differences in the methodologies. The plasma assay utilized a sequential incubation (antibody first) in order to improve the sensitivity of the assay. The urine assay utilized a more standard equilibrium-type incubation and a different separant, Pansorbin® cell suspension rather than PEG–GARG separant, in order to reduce the intersubject variability observed in the human urine matrix. Although both assay methods were precise and accurate, the differences in the methodology resulted in different ED_{50} values and variabilities in the slope coefficient (b) and the concentration at 50% binding (ED_{50}).

The standard curve ranges were 2.5–100 ng ml^{-1} and 5.0–1000 ng ml^{-1} in plasma and urine respectively; the r^2 values exceeded 0.996. The sensitivity of the assay proved to be very good with the LLQ being 2.5 ng ml^{-1} of plasma and 5 ng ml^{-1} (diluted 2.5-fold) of urine. Stavudine was stable in plasma for at least 4 days at room temperature and at 4°C and for at least 1 year

when stored at −20°C. Stability was also observed when the plasma and urine samples were subjected to 2–3 freeze–thaw cycles. The application of the RIA for the determination of stavudine in clinical samples following oral dosing was successfully demonstrated. As shown in Figs. 3 and 4, the data generated by RIA and HPLC are in excellent agreement. The half-life of stavudine in adult patients with HIV infection is approximately 1 h [9]. The plasma RIA assay was successful in profiling plasma concentrations of stavudine for approximately 4.5 half-lives.

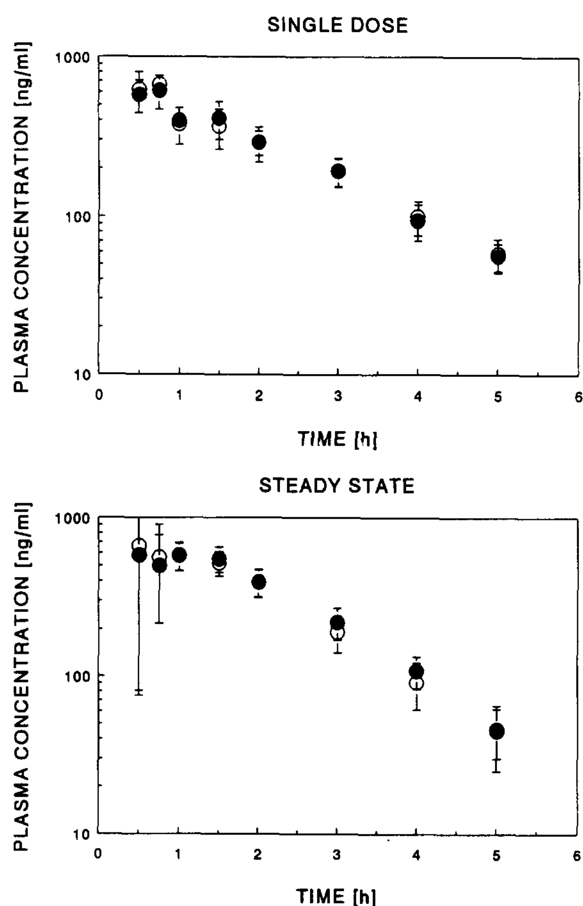

Fig. 3. Mean (SEM) plasma concentrations of stavudine in patients ($n = 3$) following oral administration of 0.67 mg kg^{-1} dose. The plasma samples were analyzed by RIA (●) and HPLC (○) assay methods.

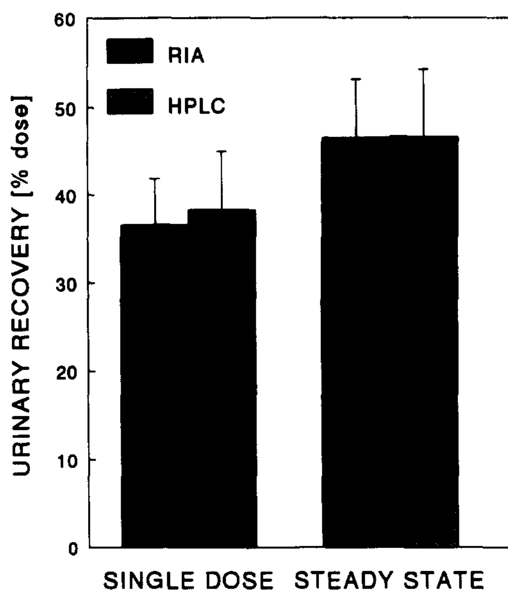

Fig. 4. Mean (SEM) urinary recovery of stavudine in patients ($n = 3$) over 8 h following oral administration of 0.67 mg kg^{-1} dose. The urine samples were analyzed by RIA and HPLC assay methods.

In conclusion, simple, specific, sensitive, precise, and accurate RIAs for the quantitation of stavudine in human plasma and urine have been developed. The LLQs were 2.5 ng of stavudine per milliliter of plasma and 5.0 ng of stavudine per milliliter of urine (diluted 2.5 fold). The assays are suitable for the evaluation of the pharmacokinetics of stavudine in humans.

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References

[1] L. Dunkle, R. Anderson, C. McLaren, A. Cross, M. Brown, R. Gugliotti, M. Adler and S. Kaul, Program of Abstracts, VIII Int. Conf. AIDS, Amsterdam, 19-24 July, 1992, Abstract WeB 1011.

[2] E. Peterson, C. Ramirez-Ronda, R. Schwartz, D. Peterson, W. Hardy, H. Sacks and S. Follansbee, Program of Abstracts, VIII Int. Conf. AIDS, Amsterdam, 19-24 July, 1992, Abstract PoB 3023.

[3] M.W. Kline, L.M. Dunkle, S. Kaul, A.T. Harris, M.E. Federici, H.M. Rosenblatt, I.C. Hanson and W.T. Shearer, Program of Abstracts, 1st National Conf. Human Retroviruses, Washington, DC, 12-16 December, 1993, Abstract 585.

[4] J.S. Janiszewski, D.E. Mulvana, S. Kaul, K.A. Dandekar and R.H. Barbhaiya, J. Chromatogr., 577 (1992) 151-156.

[5] R.H. Farmen, J.F. Muniak and K.A. Pittman, Drug Inf. J., 21 (1987) 141-152.

[6] A. DeLean, P.J. Munson and D. Rodbard, Am. J. Physiol., 235 (1978) E97-E102.

[7] W.J. Dixon and F.J. Massey, Introduction to Statistical Analysis, 4th edn., McGraw-Hill, New York, 1983.

[8] M.J.M. Hitchcock, Antiviral Chem. Chemother., 2 (1991) 125-132.

[9] M.N. Dudley, K.K. Graham, S. Kaul, S. Geletko, L. Dunkle, M. Browne and K. Mayer, J. Infect. Dis., 166 (1992) 480-485.