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Short Communication

Rapid and simultaneous determination of zidovudine and its glucuronide metabolite in plasma and urine. Application to the pharmacokinetic interaction of zidovudine and probenecid in the monkey

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Introduction

Quantitation of zidovudine (3′-azido-3′-deoxythymidine, AZT) in biological medium is required for determination of its pharmacokinetics, and as a means to evaluate clinical dosage regimens. Analytical methods have been reported to measure AZT, and in some cases its 5′-*O*-glucuronide (AZTG) metabolite in plasma or serum and urine samples of man and experimental animals [1–8]. Most of these procedures are able to quantitate AZT in serum utilizing liquid chromatography (LC) [1–7]. These techniques are suitable when therapeutic drug monitoring or pharmacokinetic analyses of AZT are desired. Characterization of the metabolism of AZT also requires quantitation of AZTG in plasma and urine. Information on the combined disposition of AZT and AZTG is critical to the evaluation and interpretation of the pharmacokinetic basis of drug interactions with AZT. Of the methods reported, Good *et al.* [1] and Lacroix *et al.* [7] have developed procedures to quantitate AZT and AZTG simultaneously in human serum. The latter method [7] has also been applied to AZT and AZTG in urine. The method of Good *et al.* [1] cannot be used for the analysis of AZT and AZTG in urine because of interferences from endogeneous substances. Measurement of AZT and AZTG in urine according to Blum *et al.* [5], required gradient elution with analysis times of 30 min. Simultaneous measurement of AZT and AZTG in human serum or urine by the method of Lacroix *et al.* [7] required column switching.

The monkey has been shown to exhibit pharmacokinetics of AZT most similar to man [8], and should serve as an appropriate animal model to characterize drug interactions of AZT. Because AIDS patients are exposed to multiple and variable drug combinations, monkeys permit controlled and comprehensive, intravenous and oral dosing, pharmacokinetic investigations to be conducted. The purpose of this investigation was to develop an LC method to quantitate AZT and AZTG simultaneously in monkey plasma and urine. The method would have to utilize a small plasma volume, and provide a level of quantitation necessary to investigate the pharmacokinetic basis of drug interactions with AZT.

Experimental

Chemicals

AZT and AZTG were kindly provided by Burroughs Wellcome Co. (Research Triangle Park, NC). The internal standard (3′-azido-2′,3′-dideoxyuridine, AZddU) was provided by Dr David Chu, University of Georgia (Athens, GA, USA).

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HPLC grade acetonitrile and methanol were purchased from J.T. Baker (Phillipsburg, NJ, USA). All other chemicals were of analytical grade.

Sample preparation

To 200 μl plasma, 20 μl of an AZddU (0.1 mg ml^{-1}) solution was added, vortexed, and then applied to a Bond–Elut® C_{18} cartridge (3 cc, Analytichem International, Harbor City, CA, USA) previously activated with 3.3 ml of methanol, followed by 2 × 3.3 ml of phosphate-buffered saline (PBS), pH 7.2. The sample-loaded cartridges were rinsed with 2 ml PBS, and the adsorbed analytes were eluted with 2 × 1 ml of methanol. The eluted samples were evaporated to dryness under a stream of nitrogen at 50°C. The residue was reconstituted with 200 μl of the mobile phase and aliquots were injected into the chromatograph.

Urine was filtered through a 0.22-μm filter unit (Millex-GS, Millipore Products Division, Bedford, MA, USA). A 1:50 (v/v) dilution of filtered urine was prepared by adding 10 μl of a 1 mg ml^{-1} AZddU solution and 970 μl of deionized water to 20 μl of urine. A 20-μl aliquot of the final mixture was injected into the chromatograph.

Chromatography

The LC system consisted of a Waters liquid chromatograph (Water Chromatograph Division, Milford, MA, USA) including a model 712 WISP autoinjector, a model 510 pump (set at 2 ml min^{-1}), a model 484 UV-vis absorbance detector (set at 267 nm) and a model 746 data module. An analytical column (Hypersil ODS, 5 μm, 150 × 4.6 mm i.d. Alltech Assoc., Deerfield, IL, USA) preceded by a Guard-Pak® (Waters Chromatography Division, Milford, MA, USA) precolumn module containing a Bondapak® C_{18} cartridge was used for all analyses. The mobile phase consisted of acetonitrile–water (8:92, v/v) adjusted to an apparent pH of 2.5 with phosphoric acid. Peak height ratios of AZT/AZddU or AZTG/AZddU were used to calculate sample concentrations from regression equations obtained from standards prepared in blank plasma or urine. Standards were prepared by adding known amounts of AZT and AZTG to blank plasma and urine.

Results and Discussion

Chromatographic analysis

Representative chromatograms of AZT, AZTG and AZddU in monkey plasma and urine are shown in Figs 1 and 2, respectively. Retention times of AZT, AZTG and AZddU were approximately 9.5, 7.4 and 5 min, respectively. Chromatographic separations of these compounds in plasma and urine were achieved under isocratic conditions, while previously gradient elution was required for urine analysis [5]. Similar to previous methods, a low organic modifier concentration was used. However, contrary to other procedures, a buffer was not necessary, and its addition to the mobile phase had little effect on the retention or resolution of the components of interest.

Recovery

The absolute recoveries of AZT and AZTG at different plasma concentrations are presented in Table 1. The recoveries are comparable to previously reported methods. Good *et al.* [1], using an analogous solid-phase extraction obtained recoveries of 93% for AZT and AZTG in human plasma. Good *et al.* [1] obtained different recoveries from different manufactured solid-phase extraction columns, and indicated Bond–Elut® octadecyl columns provided high recoveries. Species differences (monkey vs human) and sample volume size, 200 vs 500 μl, and a slightly modified extraction procedure may have contributed to slightly lower AZT recoveries in the present study. The current method, compared with Good *et al.* [1], used a 5-min sample equilibration time, rather than a minimum of 2 min, and twice the aqueous wash volume prior to sample elution with methanol. These modifications resulted in an increased analyte recovery compared with the method of Good *et al.* [1] when used with Bond–Elut® octadecyl cartridges in our laboratory. The recoveries of AZTG seem to indicate a concentration dependence, although the 137% recovery at 0.25 μg ml^{-1} is most likely due to interferences from endogenous substances.

Precision

Intraday precision, indicated by the relative standard deviation (RSD) for measurement of AZT and AZTG was less than 18.4% (Table 2). Comparable interday precision (Table 2) was achieved with the highest RSD being

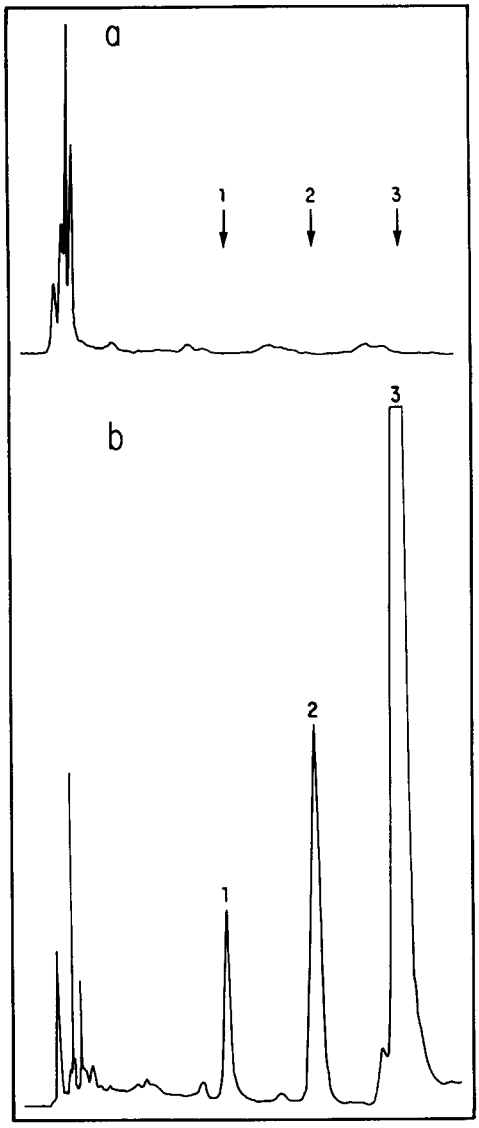

Figure 1
Chromatograms obtained from analysis of monkey plasma: (a) blank; (b) sample, 15 min post-dose, from animal administered 20 mg kg^{-1} AZT intravenously following 50 mg kg^{-1} of probenecid orally. Peak identification: 1, AZddU; 2, AZTG; and 3, AZT.

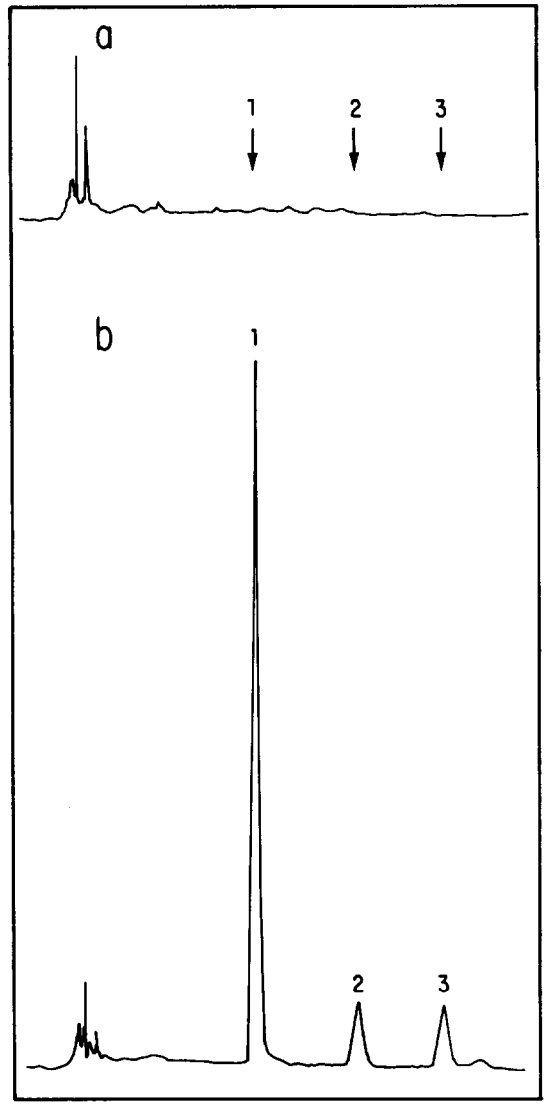

Figure 2
Chromatograms obtained from analysis of monkey urine: (a) blank; (b) sample from animal administered 20 mg kg^{-1} AZT intravenously. Peak identification: 1, AZddU; 2, AZTG; and 3, AZT.

Table 1
Absolute recovery of AZT and AZTG from monkey plasma

	AZT		AZTG	
Concentration ($\mu g\ ml^{-1}$)	Recovery* (%)	RSD† (%)	Recovery* (%)	RSD† (%)
0.25	80.8	9.2	137	7.1
2.50	88.1	16.5	93.9	13.6
25.0	72.6	10.7	71.2	11.2

*Expressed as a percentage of concentration added.
†Relative standard deviation.

Table 2
Intraday and interday precision and accuracy of AZT and AZTG analyses in monkey plasma

	AZT			AZTG		
Prepared concentration ($\mu g\ ml^{-1}$)	Measured concentration ($\mu g\ ml^{-1}$)	RSD (%)	Bias (%)	Measured concentration ($\mu g\ ml^{-1}$)	RSD (%)	Bias (%)
Intraday						
0.25	0.204	14.1	18.4	0.267	10.9	6.9
2.5	2.55	3.3	2.1	2.48	3.7	0.8
25.0	24.75	9.6	1.0	25.01	9.6	0.04
Interday						
0.5	0.485	18.9	3.0	0.475	11.5	5.0
2.5	2.47	6.3	1.2	2.45	5.2	2.2
25	24.3	2.8	2.8	24.8	3.6	0.8

$n \geq 4$.

$$\text{Bias} = \left[\frac{\text{measured concentration} - \text{prepared concentration}}{\text{prepared concentration}}\right] \times 100.$$

18.9% for AZT at 0.5 $\mu g\ ml^{-1}$. All biases were 5% or less indicating a high degree of accuracy (Table 2).

Linearity

Standard curves prepared for AZT and AZTG in monkey plasma were linear over a concentration range from 100 ng ml^{-1} to 25 $\mu g\ ml^{-1}$ ($r^2 \geq 0.998$). The mean ($n = 6$) calibration curve for AZT was, $y = 0.059 + 2.688x$, with a RSD of the slope equal to 8.8%, where y = peak height ratio and x = sample concentration. For AZTG, the mean ($n = 6$) calibration curve was $y = 0.077 + 1.526x$, with a RSD of the slope equal to 4.3%. The lower limit of quantitation in plasma was 100 ng ml^{-1} for each compound. Other procedures have reported minimum quantifiable concentrations in the order of 50 ng ml^{-1}, although this could only be achieved with larger sample volumes. Intraday and interday percentage RSDs and biases for urine analyses of AZT and AZTG were <10 and 14%, respectively. Standard curves in urine were linear from 0.4 to 20 $\mu g\ ml^{-1}$ ($r^2 \geq 0.998$). Calibration curves for AZT and AZTG in urine ($n = 6$) were $y = 0.0007 + 0.056x$ and $y = 0.0009 + 0.036x$, respectively. The RSDs for the slopes were 7.3 and 8.7% for AZT and AZTG, respectively.

Pharmacokinetic interaction of zidovudine and probenecid in the monkey

The analytical method reported here was used to characterize the pharmacokinetic basis of a drug interaction between AZT (20 mg kg^{-1}, i.v.) and probenecid (50 mg kg^{-1}, p.o.). Figure 3 illustrates AZT and AZTG plasma concentration–time profiles in the presence and absence of probenecid. Concentrations were quantitated for a minimum of 6 times the elimination half-life of AZT in the presence and absence of probenecid, indicating the

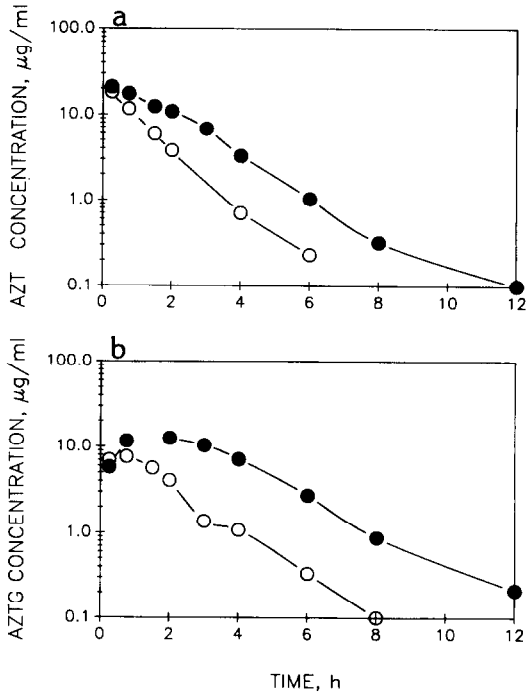

Figure 3
Semilog plot of AZT (a) and AZTG (b) concentrations in a monkey (*Macaca fasicularis*, body wt = 4.2 kg) administered 20 mg kg^{-1} of AZT intravenously alone (○), and 15 min after 50 mg kg^{-1} of probenecid orally (●). Blood samples were collected by venipuncture at the indicated times.

assay provided sufficient sensitivity. Measurement of AZT and AZTG in urine presented no problems, with milligram amounts of both substances collected over 12 h. For the data illustrated, the total clearance ($l\ h^{-1}\ kg^{-1}$) of AZT decreased from 0.79 to 0.40, as did renal clearance ($l\ h^{-1}\ kg^{-1}$) of AZT, from 0.097 to 0.038 in the presence of probenecid. Volume of distribution at steady-state declined from 0.97 to 0.77 $l\ kg^{-1}$, whereas the elimination half-life of AZT increased from 0.89 to 1.42 h in the presence of probenecid. Finally, the area under the curve for AZTG increased from 18.27 to 50.47 $\mu g\text{-}h\ ml^{-1}$ due to probenecid. Together, these findings are consistent with inhibition of AZTs glucuronidation and renal excretion by probenecid.

Conclusion

The methodology developed has been shown to be rapid and accurate for simultaneous analysis of AZT and AZTG in both plasma and urine. It does not require gradient elution or column switching, and would therefore, be a simpler and less expensive method. The method should provide an alternative method to determine the pharmacokinetics of AZT and AZTG, particularly when sample size is a limitation.

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