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MODULATION OF 3′-AZIDO-3′-DEOXYTHYMIDINE CATABOLISM BY PROBENECID AND ACETAMINOPHEN IN FRESHLY ISOLATED RAT HEPATOCYTES

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Abstract—Metabolic studies of 3′-azido-3′-deoxythymidine (AZT) in humans have demonstrated that this compound is primarily eliminated as a 5′-*O*-glucuronide, 3′-azido-3′-deoxy-5′-β-D-glucopyranuronosylthymidine (GAZT), accounting for approximately 80% of the administered dose. Recently, we characterized the complete catabolic pathway of AZT in freshly isolated rat hepatocytes in suspension, demonstrating extensive formation of three catabolites, including GAZT, 3′-amino-3′-deoxythymidine (AMT), and 3′-amino-3′-deoxy-5′-β-D-glucopyranuronosylthymidine (GAMT). The present study evaluated the effects of probenecid (PROB) and acetaminophen (ACET), two agents which are also metabolized by UDP-glucuronyltransferase, on the metabolism and transmembrane distribution of AZT in rat hepatocytes. Pre-exposure of cells to 350 μM PROB 30 min prior to the addition of 10 μM [^{3}H]AZT decreased intracellular GAZT levels by approximately 10-fold. Interestingly, AMT formation was enhanced approximately 1.5-fold in the presence of PROB, probably resulting from increased AZT availability. In contract, pre-exposure to 50 μM ACET 30 min prior to addition of 10 μM [^{3}H]AZT did not substantially alter AZT glucuronidation. Additionally, decreased AZT catabolism by PROB did not contribute to the formation of 5′-phosphorylated derivatives of AZT. Agents which undergo glucuronidation may thus not necessarily affect AZT conversion to GAZT, and their potential interactions should be investigated using *in vitro* systems prior to co-administration with AZT.

3′-Azido-3′-deoxythymidine (AZT), the only drug clinically approved for the treatment of acquired immunodeficiency syndrome (AIDS), is primarily eliminated as a 3′-azido-3′-deoxy-5′-β-D-glucopyranuronosylthymidine (GAZT) [1, 2] with approximately 80% of the administered dose in urine being identified as this catabolite [3]. Because of this extensive degradation, AZT must be given frequently in order to maintain adequate plasma concentrations. This frequency of dosing is often dependent on patient compliance and creates a major financial burden. Recently, probenecid (PROB), an inhibitor of both renal tubular secretion of organic acids and formation of acyl and ester glucuronides of a number of drugs [4, 5], was evaluated as an AZT-modulating agent in an attempt to decrease AZT degradation to GAZT. The inhibition of GAZT formation would then increase AZT plasma levels and extend its plasma elimination half-life, thereby increasing the interval over which HIV-infected cells are exposed to the drug. Studies by De Miranda *et al.* [6] and Kornhauser *et al.* [7] have demonstrated that PROB greatly alters the pharmacokinetics of AZT in humans. Mean plasma levels, area under the plasma concentration time curve (AUC), and elimination half-life ($T_{1/2}$) values of both AZT and GAZT were increased substantially following administration of AZT in the presence of PROB. In addition, the GAZT/AZT urinary excretion ratio was reduced markedly, suggesting that PROB decreased the hepatic formation and the renal excretion of GAZT. In recent studies using rhesus monkeys [8], we demonstrated that in addition to altering the pharmacokinetics of AZT and GAZT, PROB also increases the AUC and $T_{1/2}$ values of 3′-amino-3′-deoxythymidine (AMT), a novel AZT catabolite recently identified by our group in various rat and human liver systems [9] and by De Miranda and colleagues in both rat urine and feces [10]. The recent demonstration that AMT is more toxic than AZT in human bone marrow cells [9] emphasizes the need to understand the effects of PROB on the cellular disposition of AZT and its catabolites and, in particular, its effect on AMT formation. Moreover, cutaneous side-effects have been observed in some patients undergoing combined AZT/PROB administration [11, 12]; however, the significance of these clinical findings has not been elucidated yet.

Although initial studies have demonstrated that formation and excretion of GAZT in rats are minimal when compared to humans, consistent with decreased efficiency of AZT glucuronidation in rat as compared to human liver microsomes [2, 13], we recently demonstrated that GAZT is formed extensively in freshly isolated rat hepatocytes. These data would thus suggest that other factors such as recirculation, elimination in bile, or degradation by β-glucuronidase may also be involved in the quantitative differences in the amounts of GAZT excreted in urine of rats

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and humans. In addition, using this freshly isolated rat hepatocyte system, we observed rapid intracellular conversion of AZT to additional catabolites, identified as AMT, and 3′-amino-3′-deoxy-5′-β-D-glucopyranuronosythymidine (GAMT), while phosphorylation of AZT to 5′-derivatives was not detected [9].

Since previous studies on the interaction of AZT with PROB have focused primarily on the direct effects of PROB on GAZT formation and the excretion of this catabolite and AZT, the present study was designed to evaluate the effects of PROB on AZT metabolism in order to understand how these agents might interact at the cellular level. Acetaminophen (ACET), an agent which is glucuronidated extensively in humans, was also included in this study since contradictory results have been reported on the interactions between AZT and ACET [14,*–‡].

MATERIALS AND METHODS

Preparation of hepatocyte suspension. Studies were performed utilizing rat hepatocytes in suspension freshly isolated from Sprague–Dawley rats (180–200 g, Harlan Laboratories, Indianapolis, IN) by a modification of the method of Berry and Friend [15] which increases cell yield and viability [16]. Cell viability, determined by trypan blue exclusion, was ≥90% in all experiments. Hepatocytes were suspended to a final cytocrit of 10% and incubated at 37° in Krebs–Henseleit buffer containing 0.25% (w/v) gelatin and 10 mM glucose for intervals up to 2 hr. The pH was maintained at 7.4 by passing warmed and humidified 95% O_2/5% CO_2 over the cell suspension.

The cell suspension was preincubated for 30 min with 350 μM PROB or 50 μM ACET prior to the addition of [^{3}H]AZT (200 dpm/pmol) at a final concentration of 10 μM. Control incubations were exposed to 10 μM [^{3}H]AZT alone. At appropriate times, portions of the cell suspension (0.5 mL) were layered on 0.4 mL inert silicon oil (density 1.2) [16] in 1.5-mL Eppendorf microfuge tubes. The tubes were centrifuged immediately at 15,000 *g* in an Eppendorf model 5314 microcentrifuge for 30 sec and the cell pellet was frozen in dry ice/acetone. Times of incubation in the text represent the interval between addition of [^{3}H]AZT to the cell suspension and initiation of centrifugation. Portions of the extracellular medium (50 μL) were then analyzed without further processing by liquid chromatography as described below. After removing the remaining extracellular medium and aspirating the silicon oil layer, the frozen pellet was transferred to a plastic tube immersed in ice and subjected to sonic oscillation in 1 mL of 2.0 mM potassum phosphate (pH 7.4) with a 300 probe sonicator (Artex, Farmingdale, NY) for 30 sec to release intracellular [^{3}H]. The sonicate was centrifuged at 15,000 *g* at 4° in a Damon (International Equipment Co.) B-20 A centrifuge for 20 min to pellet cellular debris. Then 50–100 μL of the supernatant was analyzed by liquid chromatography. Portions (50 μL) of the extracellular and intracellular compartments were analyzed to determine total [^{3}H] in each compartment.

Analysis of intracellular and extracellular [^{3}H]-AZT metabolites by high performance liquid chromatography. Aliquots (50–100 μL) of the intracellular or extracellular compartments were analyzed by HPLC using a Hewlett–Packard model 1050 liquid chromatograph equipped with a manual injector and fixed wavelength spectrophotometer. Reversed-phase chromatography was carried out using a Hypersil ODS 5 μm column (Jones Chromatography, Littleton, CO). Elution was performed at 1 mL/min with 25 mM phosphoric acid (pH 7.2) and a 35-min linear acetonitrile gradient from 0 to 30% starting at the time of injection. Column temperature was maintained at 25° and absorbance recorded at 254 nm. Eluent from the column was directed via a low dead volume connection line into an LKB 2112 Redirac fraction collector (LKB Instruments, Rockville, MD) and timed fractions of 0.5 mL were collected into miniscintillation vials over 35 min. Radioactivity, following the addition of 5 mL of Budget-Solve scintillation fluor (Research Products International Corp., Mount Prospect, IL), was measured using a Beckman 5801 liquid scintillation counter equipped with an automatic quench correction program. Under these conditions, retention times of unlabeled authentic standards, AMT, GAZT, and AZT were 9–10, 13–14 and 21–22 min, respectively. GAMT eluted at 6–7 min and its identification has been reported recently [9]. The total radioactivity applied to the column was recovered for both extracellular and intracellular compartments in 35 min (95 ± 10% recovery based upon 72 analyses).

Determination of intracellular water. A portion (6 mL) of the hepatocyte suspension was incubated under the same conditions described above but exposed to 0.4 μCi of [*carboxyl*-^{14}C]inulin for 10–15 min to reach equilibrium between the extracellular and intracellular compartments. Portions (0.5 mL) of the cell suspension were layered onto silicon oil and centrifuged at 15,000 *g* to determine the extracellular space that accompanies cells through the oil. This value permitted corrections to be made for values of AZT and individual catabolites present in the cell pellet under these conditions. Additional samples (0.5 mL) of the cell suspension were placed and spun in preweighed Eppendorf microfuge tubes to determine intracellular volumes. Intracellular volume was the difference between the wet and dry weights of the cell pellet minus the [^{14}C]inulin space. This technique has been described previously in detail [17].

Chemicals. [5-^{3}H]AZT (3 Ci/mmol) and [*carboxyl*-

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† Koda RT, Ko RJ, Antoniskis D, Shields M and Melancon H, Effect of acetaminophen (ACET) on the pharmacokinetics of zidovudine (AZT). In: *Abstracts of the Fifth International Conference on AIDS*, p. 203, 1989.

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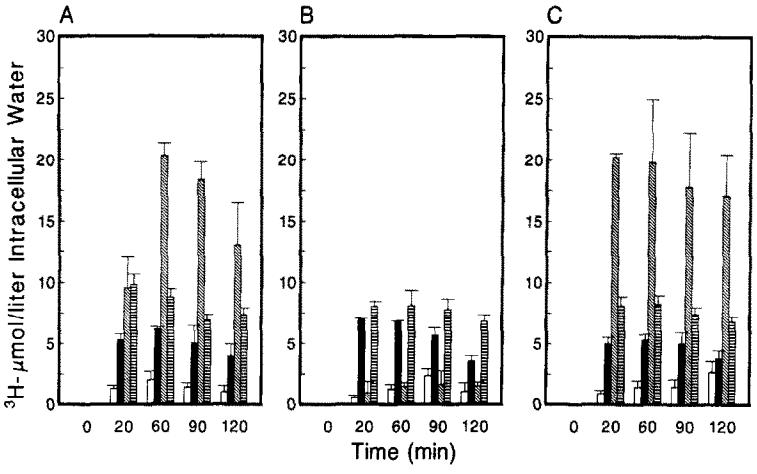

Fig. 1. Formation of unchanged AZT, GAZT, AMT, and GAMT in the intracellular water after (A) exposure of cells to 10 μM [^{3}H]AZT alone, (B) exposure of cells to 10 μM [^{3}H]AZT following a 30-min pre-exposure to 350 μM PROB, and (C) exposure of cells to 10 μM [^{3}H]AZT following a 30-min pre-exposure to 50 μM ACET. At the indicated times, portions of the cell suspension were separated by centrifugation and extracted; total intracellular [^{3}H] was analyzed by HPLC. Corrections were made from extracellular AZT and its catabolites that accompany cells through the oil. All data presented are the means of four experiments ± SD. Key: (▤) AZT, (▧) GAZT, (■) AMT, and (□) GAMT.

^{14}C]inulin were obtained from Moravek Biochemicals Inc. (City of Industry, CA) and Dupont Research Products (Wilmington, DE) respectively. [^{3}H]AZT was 99% pure as ascertained by the HPLC technique described above. Nonlabeled AZT, PROB, and ACET were purchased from the Sigma Chemical Co. (St. Louis, MO) and chemically synthesized AMT and GAZT were provided by Dr. R. F. Schinazi, Emory University and Veterans Affairs Medical Center, Decatur, GA. All other chemicals used were reagent grade.

RESULTS

Analysis of intracellular catabolites after exposure of cells to [^{3}H]*AZT and PROB or ACET.* Figure 1 illustrates the formation of GAZT, AMT and GAMT in the intracellular medium after incubation of a hepatocyte suspension over 2 hr with either 10 μM AZT alone (A), or in the presence of 350 μM PROB (B), or 50 μM ACET (C). As demonstrated recently [9], GAZT was the predominant catabolite detected in cells incubated with 10 μM AZT alone, reaching a maximum steady-state level of 20.4 ± 1.10 μM by 60 min and subsequently slowly declined. By 20 min, AMT and GAMT were detected, reaching intracellular levels of 5.32 ± 0.48 and 2.1 ± 0.3 μM, respectively. AMT then declined although one-half of the intracellular peak levels of AMT was still detected after 2 hr of incubation. Unmetabolized AZT reached a peak level of 9.86 ± 0.82 μM by 20 min and equaled those of the extracellular compartment after 2 hr, consistent with the demonstration that AZT crosses biological membranes by passive diffusion [18].

Following the addition of PROB to the incubation medium, a marked net decrease in GAZT levels (Fig. 1B) (between 0.98 ± 0.12 at 20 min and 1.73 ± 0.42 μM by 2 hr) was observed. This 10-fold reduction in the formation of GAZT reflects inhibition of AZT glucuronidation by PROB. Concurrently, AMT levels increased approximately 1.3- to 1.5-fold in the presence of PROB as compared to control. GAMT, a minor metabolite of AZT [8, 9], decreased about 50% over the first hour of exposure and returned to control values for the remainder of the experiment. The intracellular levels of unmetabolized AZT were virtually unchanged as compared to control. In contrast to PROB, the addition of ACET to the incubation medium, as illustrated in Fig. 1C, had no substantial effects on AZT or catabolite intracellular levels as compared to control (Fig. 1A). It is further interesting to note that although AZT catabolism was decreased in the presence of PROB, thus increasing AZT availability for anabolism, no nucleotide derivatives of AZT were detected within cells.

Analysis of extracellular AZT and catabolites following exposure of cells to [^{3}H]*AZT and PROB or ACET.* Analysis of the decline of extracellular AZT and accumulation of its catabolites over 20 min to 2 hr after exposure of hepatocytes to 10 μM AZT alone and in the presence of 350 μM PROB or 50 μM ACET is illustrated in Fig. 2. The changes in the levels of AZT catabolites in the extracellular compartment correlated with changes in the intracellular compartment. Unmetabolized AZT decreased at a rate of 0.053 ± 0.01 μM/min under control conditions. In contrast, no significant decrease of extracellular AZT was detected with PROB. The levels of extracellular GAZT were reduced markedly in the presence of PROB, consistent with inhibition of AZT glucuronidation within the cell. Whereas GAZT achieved a maximum extracellular concentration of 4.99 ± 0.479 μM by 2 hr under control conditions, only 0.41 ± 0.02 μM

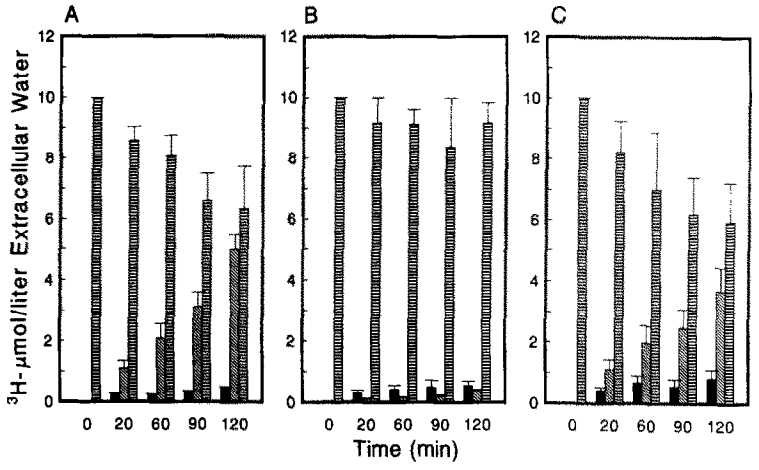

Fig. 2. Analysis of extracellular AZT and its catabolites, GAZT and AMT, after (A) exposure of cells to 10 μM [^{3}H]AZT alone, (B) exposure of cells to 10 μM [^{3}H]AZT following a 30-min pre-exposure to 350 μM PROB, and (C) exposure of cells to 10 μM [^{3}H]AZT following a 30-min pre-exposure to 50 μM ACET. At the indicated time, portions of the cell suspension were separated by centrifugation and the total [^{3}H] was assayed by HPLC. All data presented are the means of four experiments ± SD. Key: (▤) AZT, (▨) GAZT, and (■) AMT.

extracellular GAZT was observed in the presence of PROB at the same time period. Of note, the magnitude of reduction of GAZT extracellular levels correlated with the degree of inhibition of GAZT formation within cells. As detected in the intracellular compartment, AMT extracellular levels increased almost 1.5-fold in the presence of PROB.

Similar to what was demonstrated within the cell, ACET had no effect on the extracellular levels of AZT and its catabolites with essentially the same pattern observed in the control.

DISCUSSION

This study provides the first detailed analysis of the alteration of AZT catabolism by other drugs in a freshly isolated rat hepatocyte system. As recently reported [9], GAZT was the major intracellular constituent when cells were exposed to AZT alone. When a therapeutically relevant concentration of 350 μM PROB [19] was added to the incubation medium, intracellular GAZT decreased approximately 10-fold with a corresponding reduction in extracellular levels, indicating that PROB competes with AZT metabolism without effects on AZT or GAZT transport. These data are consistent with studies using microsomes which demonstrated that PROB is a strong inhibitor of AZT glucuronidation.* In contrast, formation of AMT, a cytotoxic catabolite of AZT [9], appeared to be enhanced 1.5-fold in the presence of PROB. This elevation in AMT levels by PROB does not involve a direct effect on the formation of AMT, but rather an indirect effect: in the presence of PROB, glucuronidation is essentially blocked which increases AZT availability for reduction hence enhancing AMT formation. Relevant to the present report, recent studies by our group in rhesus monkeys [8] demonstrated that monkeys which received PROB in combination with AZT had increased AMT plasma levels, possibly related to enhanced AMT formation due to increased AZT levels in plasma.

GAMT, a minor AZT catabolite [8, 9], was also detected within cells. In the presence of PROB, GAMT levels decreased approximately 50% over the first hour of exposure. Since GAMT is a reductive product of GAZT rather than a glucuronide conjugate of AMT [9], this phenomenon reflects the decreased availability of GAZT within the cell. At later time points, intracellular GAMT levels returned to control values, although only a 2-fold increase of GAZT levels was observed, suggesting that GAMT formation is rapidly saturable in liver cells. On the other hand, AZT anabolism was not observed in the presence of PROB in spite of increased AZT availability for phosphorylation.

Co-administration of ACET and AZT has been implicated in enhancing AZT-induced myelosuppression [14,†,‡]. Early *in vitro* studies using liver microsomes* indicated that ACET is a weak inhibitor of AZT glucuronidation when compared to PROB, suggesting that competition for glucuronidation by UDP-glucuronyltransferase (UDPGT) is possible and may be responsible for the enhancement of AZT toxic side-effects observed when ACET is administered together with AZT

* Unadkat JD, Schumann L and Roskos LA, Simple and rapid *in vitro* microsomal system to screen for metabolic drug–drug interactions with anti-HIV compounds. In: *Program and Abstracts of Antimicrobial Agents and Chemotherapy*, p. 345, 1988.

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[14,*,†]. However, in the present study, addition of a clinically relevant concentration of 50 μM ACET [19] to the hepatocyte suspension had no significant effect on the formation of GAZT, AMT or GAMT. Furthermore, *in vivo* studies have demonstrated that concomitant use of AZT and ACET had no effect either on the disposition of GAZT or on the pharmacokinetics of AZT [14,*,†]. Thus, our present study further demonstrates that enhanced AZT toxicity observed with co-administration of ACET and AZT does not occur through inhibition of AZT glucuronidation but rather by a different mechanism.

The findings presented here, combined with the findings of previously reported studies both in human and liver microsomal preparations [9], further define the *in vitro* catabolism of AZT in intact cells and the effects of the modulating agents PROB and ACET which are two drugs known to undergo glucuronidation. Thus, these agents may potentially interfere with the elimination of AZT through inhibition of GAZT formation. Interestingly, ACET had no effect on AZT glucuronidation, while PROB affected GAZT, AMT and GAMT formation. One explanation for the differential abilities between ACET and PROB to inhibit GAZT formation may reflect a different glucuronidation process through various UDPGT isoenzymes. Previous studies have indeed shown that rat and human liver contain multiple forms of UDPGT [20, 21]. In fact, two UDPGT isoenzymes which are involved in xenobiotic glucuronidation have been isolated and purified from human liver [20, 21]. Thus, it is quite likely that PROB and AZT use the same UDPGT isoenzyme for glucuronidation while ACET uses another. This suggests that other agents which undergo glucuronidation may not necessarily affect GAZT formation and, therefore, potential interactions between AZT and agents which are eliminated *in vivo* through glucuronidation need to be investigated using relevant hepatic *in vitro* systems.

Although direct extrapolation of *in vitro* animal studies to the clinical situation should be made with caution, it should be emphasized that consistent findings on the AZT/PROB interaction have been observed *in vivo*, using rhesus monkeys [8], an appropriate animal model for AZT disposition and metabolism [22, 23]. These data suggest that combination therapy of AZT and PROB would result in the enhanced formation of AMT, potentially resulting in increased AZT side-effects.

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REFERENCES

1. Good SS, Koble CS, Crouch R, Johnson RL, Rideout JL and de Miranda P, Isolation and characterization of an ether glucuronide of zidovudine, a major metabolite in monkeys and humans. *Drug Metab Dispos* **18**: 321–326, 1990.
2. Cretton EM, Waterhous DV, Bevan R and Sommadossi J-P, Glucuronidation of 3′-azido-3′-deoxythymidine by rat and human liver microsomes. *Drug Metab Dispos* **18**: 369–372, 1990.
3. Blum MR, Liao SHT, Good SS and de Miranda P, Pharmacokinetics and bioavailability of zidovudine in humans. *Am J Med* **85**: 189–194, 1988.
4. Smith PC, Lanjendijk PN, Bosso JA and Benet LZ, Effect of probenecid on the formation and elimination of acyl glucuronides: Studies with zomepirac. *Clin Pharmacol Ther* **38**: 121–127, 1985.
5. Abernethy DR, Greenblatt DJ, Ameer B and Snader RJ, Probenecid impairment of acetaminophen and lorazepam clearance: Direct inhibition of ether glucuronide formation. *J Pharmacol Exp Ther* **234**: 345–349, 1985.
6. De Miranda P, Good SS, Yarchoan R, Thomas RV, Blum MR, Charles E and Broder S, Alteration of zidovudine pharmacokinetics by probenecid in patients with AIDS and AIDS-related complex. *Clin Pharmacol Ther* **45**: 473–475, 1989.
7. Kornhauser DM, Hendrix CW, Nerhood LJ, Petty BG, Woods AS, Bartlett JG and Lietman PS, Probenecid and zidovudine metabolism. *Lancet* **II**: 473–475, 1989.
8. Cretton EM, Schinazi RF, McClure HM, Anderson DC and Sommadossi JP, Pharmacokinetics of 3′-azido-3′-deoxythymidine and its catabolites and interactions with probenecid in rhesus monkeys. *Antimicrob Agents Chemother* **35**: 801–807, 1991.
9. Cretton EM, Xie M-Y, Bevan RJ, Goodgaon NM, Schinazi, RF and Sommadossi J-P, Catabolism of 3′-azido-3′-deoxythymidine in hepatocytes and liver microsomes with evidence of formation of 3′-amino-3′-deoxythymidine, a highly toxic catabolite for human bone marrow cells. *Mol Pharmacol* **39**: 258–266, 1991.
10. De Miranda P, Burnette TC and Good SS, Tissue distribution and metabolic disposition of zidovudine in rats. *Drug Metab Dispos* **18**: 315–320, 1990.
11. Duckworth AS, Duckworth GW, Henderson G and Contreras G, Zidovudine with probenecid. *Lancet* **336**: 441, 1990.
12. Petty BG, Kornhauser DM and Lietman PS, Zidovudine with probenecid: A warning. *Lancet* **335**: 1044–1045, 1990.
13. Reseter A and Spector T, Glucuronidation of 3′-azido-3′-deoxythymidine: Human and rat enzyme specificity. *Biochem Pharmacol* **38**: 1389–1393, 1989.
14. Steffe EM, King JH, Inciardi JF, Flynn NF, Goldstein EG, Tonjes TS and Benet LZ, The effect of acetaminophen on zidovudine metabolism in HIV-infected patients. *J Acquir Immun Defic Syndr* **3**: 691–694, 1990.
15. Berry MN and Friend DS, High yield preparation of isolated rat liver parencymal cells: A biochemical and fine structural study. *J Cell Biol* **43**: 506–520, 1969.
16. Sommadossi J-P, Gewirtz DA, Diasio RB, Aubert B, Cano JP and Goldman ID, Rapid catabolism of 5-fluouracil in freshly isolated rat hepatocytes as analyzed by high performance liquid chromatography. *J Biol Chem* **257**: 8171–8176, 1982.
17. Cybulski RL, Fry DW and Goldman ID, Adenosine stimulation of uphill adenine transport in L1210 leukemia cells. *J Biol Chem* **256**: 4455–4459, 1981.
18. Zimmerman TP, Mahony WB and Prus L, 3′-Azido-3′-deoxythymidine: An unusual nucleoside analogue

that penetrates the membrane of human erythrocytes and lymphocytes by nonfacilitated diffusion. *J Biol Chem* **262**: 5748–5754, 1987.
19. *Drug Information for the Health Care Provider USPDI*, 7th Edn, Vol. 1. Mark Printing Co., Easton, PA, 1987.
20. Chowdhury JR, Chowdhury MR, Falany CN, Tephly TR and Arias IM, Isolation and characterization of multiple forms of rat liver UDP-glucuronate glucuronosyltransferase. *Biochem J* **233**: 827–837, 1986.
21. Irshaid YM and Tephly TR, Isolation and purification of two human liver UDP-glucuronosyltransferases. *Mol Pharmacol* **31**: 27–34, 1987.
22. Boudinot FD, Schinazi RF, Gallo TM, McClure HM, Anderson DC, Doshi KJ, Kambhampthi PC and Chu CK, 3′-Azido-3′-deoxyuridine (AzddU): Comparative pharmacokinetics with 3′-azido-3′-deoxythymidine (AZT) in monkeys. *AIDS Res Hum Retroviruses* **6**: 219–220, 1990.
23. De Miranda PT, Burnett C and Good SS, Disposition and pharmacokinetics of the antiviral drug 3′-azido-3′-deoxythymidine (Retrovir) in monkeys and rats. In: *Abstracts of the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy* p. 372. American Society of Microbiology, New York, 1987.