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METABOLISM OF 3′-AZIDO-3′-DEOXYTHYMIDINE (AZT) IN HUMAN PLACENTAL TROPHOBLASTS AND HOFBAUER CELLS

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Abstract—3′-Azido-3′-deoxythymidine (AZT) is currently under clinical investigation to assess its potential to inhibit maternal–fetal HIV transmission. To determine the activation of AZT to its phosphorylated metabolites by placental cells, we characterized the intracellular phosphorylation of AZT in two major cell types of the placenta, namely trophoblasts and Hofbauer cells. Although phosphorylation of AZT in trophoblast and Hofbauer cells is 50- to 100-fold lower than that in human lymphocytic cell lines or activated lymphocytes, both cell types are capable of activating AZT to AZT triphosphate (AZTTP) at a level comparable to that of resting lymphocytes. We found that AZT monophosphate (AZTMP) was the major phosphorylated AZT metabolite, while AZT diphosphate (AZTDP) and AZTTP constituted less than 4% of the intracellular phosphorylated AZT pool. This result was independent of AZT concentration and exposure time in both types of placental cells. The rate-limiting step in the conversion of AZT to AZTTP was determined to be thymidylate kinase-catalyzed conversion of AZTMP to AZTDP. Trophoblasts and Hofbauer cells exhibited different time-course and concentration-dependent profiles of intracellular AZT phosphorylation, suggesting that these two placental cells may have anabolic or catabolic enzymes of different composition or efficiency. AZTTP decayed in both trophoblasts and Hofbauer cells with a half-life of 4–6 hr. These results should be useful in rationally designing AZT dosage regimens to treat HIV-infected women for prevention of maternal–fetal HIV transmission.

Key words: azidothymidine; phosphorylation; Hofbauer cells; trophoblasts; placenta; zidovudine; maternal–fetal HIV transmission; progesterone

Women and children represent a rapidly increasing proportion of individuals infected with HIV‡. Although the exact mode of transmission is unclear, it is estimated that perinatal transmission accounts for 80% of pediatric cases of AIDS [1]. Localization of HIV antigen p24 and HIV nucleic acid sequences in the placental trophoblasts and Hofbauer cells (placental macrophages) of HIV-infected women indicates that in utero HIV infection of the fetus is possible [2]. In addition, HIV infection can also occur at a late gestational age of pregnancy and/or delivery [3, 4]. Trophoblasts and Hofbauer cells are two major types of cells found in the placenta. Trophoblasts, metabolically the most active cells of the placenta, are involved in placental growth, metabolism and transport of nutrients, and are also responsible for maintaining pregnancy by hormonal secretions. The function of Hofbauer cells is unclear at present; however, they are not known to synthesize placental hormones. As placental trophoblasts and Hofbauer cells are exposed continuously to maternal uterine decidua and blood, these cells may play an important role in HIV transmission from the mother to the fetus, an event that may be inhibited by antiviral therapy.

AZT (zidovudine, azidothymidine) is the first drug approved for the treatment of AIDS. Recent studies indicate that the parent drug is able to cross the placenta [5–7] and achieve therapeutic concentrations in the fetus [8, 9]. To determine the therapeutic effect of AZT on maternal–fetal HIV transmission, a large, randomized, placebo-controlled clinical trial (ACTG 076) is in progress. The results of perfused placental studies indicate that a significant amount of AZT retained in the tissue is likely to be the phosphorylated AZT metabolites [6]. However, these AZT metabolites have, as yet, not been characterized.

AZT enters blood cells by diffusion [10], where it is phosphorylated to AZTMP, AZTDP and AZTTP by cellular kinases [11]. AZTTP has been shown to interfere with HIV replication through inhibition of viral reverse transcriptase and, therefore, the inhibition of proviral DNA synthesis [11–13]. Also, AZTMP may play a role in inhibiting HIV viral replication through inhibition of the RNase H activity of HIV reverse transcriptase [14]. At a high concentration, both AZTTP and AZTMP can

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‡ Abbreviations: HIV, human immunodeficiency virus; AZT, 3′-azido-3′-deoxythymidine; AIDS, acquired immunodeficiency syndrome; AZTMP, AZT-monophosphate; AZTDP, AZT-diphosphate; AZTTP, AZT-triphosphate; FBS, fetal bovine serum; and $T_{1/2\beta}$, terminal elimination half-life.

inhibit human cellular DNA polymerase [15, 16]. Phosphorylated AZT metabolites have been shown to interfere with cellular DNA synthesis in the bone marrow cell [17]. Recently, AZT has been shown to inhibit DNA synthesis and hormone secretion by human placental trophoblasts [18]. However, the relationship between this inhibitory effect of AZT and its intracellular phosphorylation profile remains to be elucidated.

Because AZT must be phosphorylated to produce its antiviral effect, understanding the kinetics and the extent of its phosphorylation in placental cells may aid in optimizing AZT drug therapy for HIV-infected pregnant women while minimizing the toxicity of the drug. Therefore, we investigated the time- and concentration-dependent phosphorylation of AZT in human placental trophoblasts and Hofbauer cells.

MATERIALS AND METHODS

Chemicals. 3′-Azido[*methyl*-^{3}H]-3′-deoxythymidine ([^{3}H]AZT, radiochemical purity, >99.7% 14 Ci/mmol) was purchased from Moravek Biochemical (Brea, CA). AZTMP, AZTDP and AZTTP were obtained from the Burroughs Wellcome Co. (Research Triangle Park, NC). [^{3}H]dT (84 Ci/mmol) was purchased from NEN (Wilmington, DE). FBS was purchased from Hyclone (Logan, UT). RPMI 1640 and antibiotics were purchased from Gibco BRL (Grand Island, NY). All other chemical and reagents were of analytical or high performance liquid chromotographic (HPLC) grades.

Isolation of trophoblasts and Hofbauer cells. Human term placentas from HIV seronegative mothers were collected following normal term delivery. All the placentas collected were processed immediately, as described previously [18]. Briefly, the placenta villi were digested with dispase enzyme (25 enzyme units/g tissue) to obtain a single cell suspension. These placental cells were loaded on a two-step Percoll gradient containing 10 mL each of 29% (w/v) (d = 1.057 g/mL) and 48% (w/v) (d = 1.066 g/mL) Percoll to isolate trophoblasts and Hofbauer cells. Placental cells loaded onto the two-step gradients in 50 mL conical centrifuge tubes were centrifuged at 400 *g* for 25 min to separate trophoblast, Hofbauer, and red cells. After discarding the red cell fraction, the remaining cells were washed separately three times in RPMI 1640–1% fetal bovine serum and plated into tissue culture plates at 5–10 × 10^6 cells in 1–2 mL of RPMI with 10% FBS.

Cell culture and incubation with AZT. Trophoblasts and Hofbauer cells (5–10 × 10^6 cells/mL) were incubated separately in RPMI 1640 medium that contained 10% FBS, supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL fungizone (Gibco BRL). After the addition of [^{3}H]-AZT at indicated concentrations to the medium, these cells were maintained at 37° in a 5% CO_2 incubator.

Effects of AZT on DNA synthesis and progesterone production of trophoblasts. The synthesis of DNA by placental trophoblasts was evaluated by the ability of cells to incorporate [^{3}H]dT. We have shown previously that the inhibitory effect of AZT on DNA synthesis in trophoblasts was similar, irrespective of whether [^{3}H]dT or [^{14}C]dU was used to detect the degree of DNA synthesis [18]. On day 2 of incubation, the trophoblasts were pulsed with 5 μCi of [^{3}H]dT for 18 hr, and the cellular DNA was collected onto a glass fiber using a cell harvester (Cambridge Tech., Watertown, MA). The degree of cell DNA synthesis was determined by scintillation counting of [^{3}H]dT incorporated into cellular DNA. Inhibition of DNA synthesis by AZT was determined for three placentas in quadruplicates, and the data were expressed as mean percent inhibition. Inter- and intra-placental variations for these values were less than 15% of the mean.

The effect of AZT on progesterone production by placental trophoblasts was assessed by determining the concentration of the hormone in the supernatant (100 μL) of the cells on day 3 by HPLC, as described previously [18]. The effect of AZT on progesterone production is expressed as mean percent inhibition (compared with that without AZT) of three placentas. Intra-placental variation was less than 20%.

Preparation of cell extracts to analyze phosphorylated metabolites. At indicated time points, the cells were scraped from the tissue culture plate and washed twice with 10 mL of ice-cold phosphate buffer solution (pH 7.4) by centrifugation (300 *g*) at 4° for 5 min. Aliquots of the incubation medium were collected and stored at −20° for further analysis. To minimize degradation of the nucleotides, 300 μL of cold (−20°) acetonitrile was added to the cell pellets to precipitate the macromolecules, followed within 2 min by the addition of 200 μL of ice-cold distilled water to extract the cellular content. The extracts were centrifuged at 12,000 *g* for 1 min to remove cell debris; the supernatants were transferred to a microcentrifuge tube and evaporated to dryness under a gentle stream of nitrogen gas. Previous stability studies with [^{3}H]AZTTP showed that it is chemically stable under the above extraction procedure. All samples were stored at −20° until analysis by HPLC.

Analysis of [^{3}H]*AZT metabolites by HPLC.* AZT nucleotides were separated using a linear gradient HPLC system equipped with a Partisil SAX anion exchange column (Whatman, NJ). The mobile phases consisted of two components: A (10% methanol) and B (0.25 M $NH_4H_2PO_4$ and 1 M KCl, pH 4.0). Sample was eluted at 2.5 mL/min as follows: (1) at time 0–3 min, 100% component A; (2) at 3 min, component B was increased linearly from 0 to 100% over 20 min; (3) at 23–50 min, 100% B. The eluant was collected with a fraction collector at 2-min intervals (5 mL/vial), mixed with 15 mL of scintillation fluid Aquasol II (Du Pont, MA), and counted on a liquid scintillation analyzer Tri-Carb 2000 (Packard, IL). Under our experimental conditions, retention times of AZT, AZTMP, AZTDP, and AZTTP standards were 1.9, 11, 20, and 37 min, respectively. The corresponding radiolabeled peaks were assigned based on retention times. The reproducibility of this assay was examined using CEM cells incubated with 1.79 μM [^{3}H]AZT for 4 hr, and the intracellular metabolites were

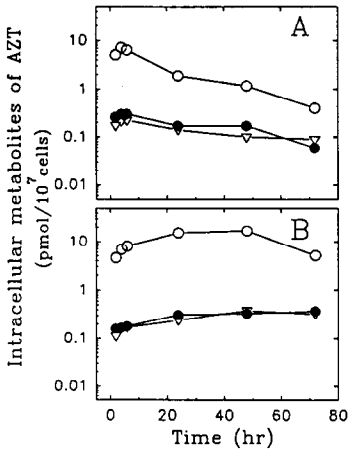

Fig. 1. Time course of AZT phosphorylation in human placental cells. Primary human trophoblasts (A) or Hofbauer cells (B) were incubated with 1.79 μM [^{3}H]AZT (14 Ci/mmol), and, at the indicated time, intracellular accumulation of AZTMP (○), AZTDP (●), and AZTTP (▽) was determined as pmol/10^7 cells.

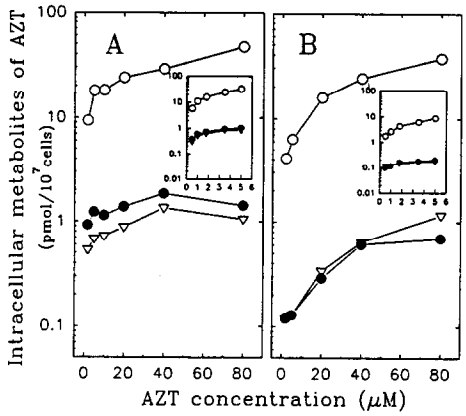

Fig. 2. Effect of AZT concentration on intracellular accumulation of phosphorylated AZT. Human placental cells—trophoblasts (A) or Hofbauer cells (B)—were incubated with indicated concentrations of AZT for 4 hr. Accumulation of intracellular AZTMP (○), AZTDP (●), and AZTTP (▽) was determined. Panels A and B show results with AZT concentrations ranging from 1.79 to 80 μM. Insets: results of 0.5 to 5 μM AZT concentration range using cells isolated from a different placenta.

extracted and analyzed. The coefficient of variation values for the intracellular levels of AZTMP, AZTDP, and AZTTP, determined in quadruplicate, were 11.1, 10.1 and 8.8%, respectively.

Decay kinetics of AZT and its metabolites in trophoblasts and Hofbauer cells. To determine the decay kinetics of phosphorylated AZT in placental cells, trophoblasts and Hofbauer cells were pre-incubated with 10 μM [^{3}H]AZT for 4 hr. The drug was removed by centrifugation, and the cells were washed three times with 12 mL of prewarmed medium. The cells then were resuspended in fresh, drug-free medium and incubated at 37° 5% CO_2. At 0, 0.5, 1, 2, 4, 6, and 12 hr after AZT removal, the intracellular levels of phosphorylated AZT metabolites were determined, and the intracellular level versus time data were analyzed using nonlinear regression.

Correlation of AZT metabolism with cellular DNA synthesis and progesterone production. Intracellular concentrations of AZTTP metabolites were correlated with AZT inhibitory effect (%) on either DNA synthesis or progesterone production in placental trophoblasts. The concentration of intracellular AZTTP at half the maximum effect was estimated from the data by fitting the sigmoid E_{max} model [19] to the data using the Sigmaplot curve fitting program (Jandel Scientific, Corte Madera, CA).

RESULTS

Kinetics of intracellular AZT phosphorylation in trophoblasts and Hofbauer cells. The time course of AZT phosphorylation in trophoblasts and Hofbauer cells was determined by incubating the placental cells with 1.79 μM [^{3}H]AZT for 2–72 hr. As shown in Fig. 1, AZT was converted rapidly to AZTMP in placental cells, and AZTMP constituted the major metabolic species (>70%) of the total phosphorylated metabolites in trophoblasts and Hofbauer cells. In trophoblasts, intracellular AZTMP, AZTDP, and AZTTP levels peaked within 4–6 hr. The intracellular level of AZTMP declined markedly beyond 4 hr of incubation (Fig. 1A). The levels of AZTDP and AZTTP remained low, and a small decrease was seen for these metabolites after 4 hr.

A different time course of AZT phosphorylation was observed in Hofbauer cells (Fig. 1B). The rise in the AZTMP level was more gradual, and no significant drop-off was seen up to 48 hr of incubation. In parallel, AZTDP and AZTTP levels followed the trend of AZTMP kinetics. Although the peak level of AZTMP was about 2.3-fold higher in Hofbauer cells than in trophoblasts, AZTDP and AZTTP levels in the two placental cells were not significantly different. Overall, trophoblasts and Hofbauer cells showed some difference in phosphorylation of AZT, but they both were capable of forming limited amounts of AZTTP.

Effect of AZT concentration on intracellular AZT phosphorylation in trophoblasts and Hofbauer cells. To determine the concentration effect of AZT on its intracellular metabolism, trophoblasts and Hofbauer cells were incubated with various concentrations of AZT for 4 hr, and the distribution of phosphorylated AZT in these cells was determined. The 4-hr time point was chosen because at that time, a maximum accumulation of intracellular AZT metabolites was observed (Fig. 1). In trophoblasts, an increasing concentration of extracellular AZT (from 1.79 to 80 μM) resulted in a 5-fold increase of the intracellular AZTMP level (from 9.4 to 46.7 pmol/10^7 cells), while the increase in either AZTDP or AZTTP was less than 2-fold under the same conditions (Fig. 2A).

In Hofbauer cells, a similar increase in the AZTMP

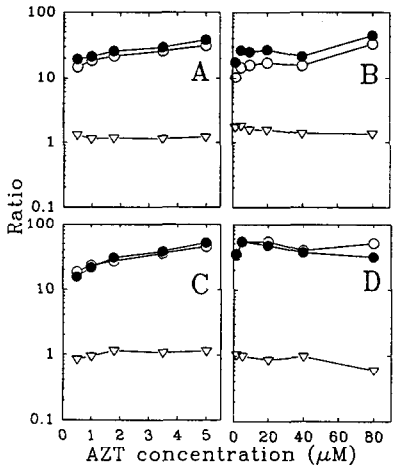

Fig. 3. Ratios of AZT phosphorylated metabolites in human placental cells as a function of AZT concentration. The results of the two separate experiments presented in Fig. 2 were analyzed as the ratios of AZTMP/AZTDP (○), AZTMP/AZTTP (●), and AZTDP/AZTTP (▽) for trophoblasts (A and B) and Hofbauer cells (C and D).

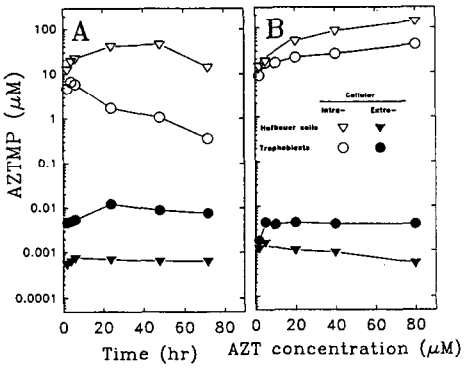

Fig. 4. Comparison of intracellular and extracellular accumulation of AZTMP in human placental cells. Intracellular AZTMP concentrations in human trophoblasts (○) and Hofbauer cells (▽) and their respective extracellular concentrations (●, ▼) were determined as described in Materials and Methods. The results are presented as a function of time (A) and a function of AZT concentration (B).

level was also detected with the extracellular AZT concentration ranging from 1.79 to 80 μM. However, AZTDP and AZTTP levels in these cells exhibited a 6- to 10-fold increase (instead of 2-fold for trophoblasts) over the same AZT concentration range (Fig. 2B). For both types of placental cells, the studies with lower AZT concentrations (0.5 to 5 μM) produced a metabolism profile (Fig. 2 insets) consistent with that observed with a higher concentration of AZT (Fig. 2, A and B).

To characterize further the differences in the efficiency of AZT phosphorylation in the placental cells, we compared the ratio of AZT metabolites as AZTDP/AZTTP, AZTMP/AZTDP, and AZTMP/AZTTP. The AZTMP/AZTDP and AZTMP/AZTTP ratios for trophoblasts were slightly lower than those of Hofbauer cells at the lower AZT concentration (0.5 to 5 μM) (Fig. 3, A and C) and 2- to 3-fold lower for the higher AZT concentration (1.79 to 80 μM) (Fig. 3, B and D). In contrast, AZTDP/AZTTP ratios for trophoblasts were about 1.5-fold higher than those of Hofbauer cells (Fig. 3, B and D).

Determination of AZT phosphorylated metabolites in the culture medium. As AZTMP has been shown to be released into the growth medium of lymphocytic cells, we also determined extracellular concentrations of AZT metabolites for trophoblasts and Hofbauer cells with respect to AZT concentration and time of incubation. With a 2-hr-exposure of extracellular AZT at 1.79 μM, AZTMP was detected in the medium of both trophoblasts and Hofbauer cells. The AZTMP detected in the culture medium was not due to cell death, as determined by cell morphology and trypan blue exclusion analysis. Time and AZT concentration effects on intra- and extracellular AZTMP concentration were compared as molar concentration instead of amount of AZTMP. The molar concentration of AZTMP was calculated based on a mean packed volume of 1.1 μL/10^7 cells for trophoblasts and 0.37 μL/10^7 cells for Hofbauer cells. The extracellular concentration of AZTMP constituted less than 2% of intracellular concentrations in trophoblasts, and less than 0.001% in Hofbauer cells (Fig. 4). The time-dependent decrease in intracellular AZTMP (after 4 hr) in trophoblasts was reflected in the increase in extracellular concentration (Fig. 4A). Under the same condition, Hofbauer cells did not exhibit time-dependent decay of intracellular AZTMP (Fig. 4B). Neither AZTDP nor AZTTP was detectable in the medium of either cell line, further supporting the conclusion that cell death was probably not the cause of AZTMP being released into the extracellular medium.

Decay kinetics of AZT and its metabolites in trophoblasts and Hofbauer cells. To determine the time-dependent decay of phosphorylated AZT, trophoblasts and Hofbauer cells were pulsed with [^{3}H]AZT for 4 hr, and the decay rates of intracellular AZT and phosphorylated AZT metabolites were determined. As shown in Fig. 5, upon the removal of extracellular AZT, the intracellular AZTMP declined rapidly with a $T_{1/2\beta}$ of 3.9 and 2.5 hr for trophoblasts and Hofbauer cells, respectively. AZTDP and AZTTP decayed at a slower rate, with a $T_{1/2\beta}$ of 7.4 and 4.6 hr in trophoblasts and 4.7 and 5 hr in Hofbauer cells (Fig. 5). Hence, while the trophoblasts and Hofbauer cells exhibited different elimination rate constants for AZT and its metabolites, both types of placental cells eliminated AZT and AZTMP at a significantly higher rate than they eliminated AZTDP or AZTTP.

Effect of phosphorylated AZT concentration on DNA and progesterone synthesis in placental trophoblasts. As phosphorylated AZT in placental cells may inhibit DNA synthesis, we compared the intracellular AZTTP concentration with its inhibitory

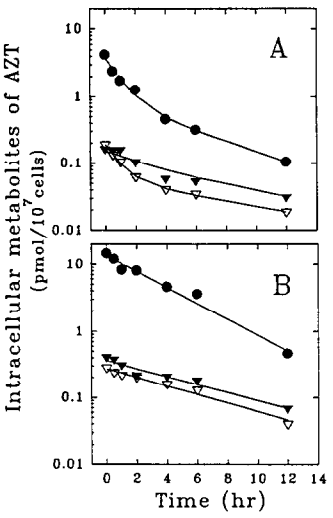

Fig. 5. Decay kinetics of AZT phosphorylated metabolites in human placental cells. Human trophoblasts (A) and Hofbauer cells (B) were incubated with 10 μM [^{3}H]AZT for 4 hr. At the indicated time, the intracellular concentrations of AZT nucleotides [AZTMP (●), AZTDP (▽), and AZTTP (▼)] were determined as described in Materials and Methods.

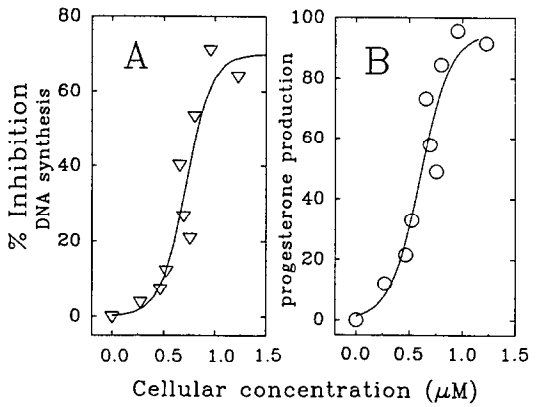

Fig. 6. Correlation of intracellular AZTTP concentration and the inhibitory effect of AZT on DNA synthesis (A) and progesterone production (B) in human trophoblasts. The predicated values obtained using nonlinear regression form the continuous line.

effect on DNA and progesterone synthesis by trophoblasts (Fig. 6). As Hofbauer cells do not synthesize a significant amount of DNA, the inhibitory effects of AZTTP cannot be discerned in these cells. AZTTP inhibited DNA synthesis, with the half-maximum inhibitory concentration (IC_{50}) estimated to be 0.83 μM. Similarly, AZTTP was determined to inhibit progesterone production with an IC_{50} of 0.62 μM.

DISCUSSION

We and others have shown that AZT diffuses across placental tissue [5, 6, 8, 9]. Recently, we have optimized a procedure to simultaneously isolate placental trophoblasts and Hofbauer cells, permitting the assessment of AZT on these cells at a cellular level. Using the human primary placental trophoblasts and Hofbauer cells, we have shown that AZT inhibits DNA synthesis and progesterone production of trophoblasts, whereas it has a minimal effect on Hofbauer cells [18]. In this work, we further characterized the effect of AZT on placental cells by studying the intracellular AZT metabolism in human placental trophoblasts and Hofbauer cells. It was found that both types of placental cells are able to phosphorylate AZT to AZTMP, AZTDP, and AZTTP. AZTMP was the major and AZTDP and AZTTP the minor phosphorylated metabolites of AZT (Figs. 1 and 2). The rate-limiting step of AZT phosphorylation is determined to be the conversion of AZTMP to AZTDP by thymidylate kinase, as apparent by significantly lower AZTDP/AZTTP ratios compared with AZTMP/AZTDP ratios.

We observed some differences between trophoblasts and Hofbauer cells in their capacity to phosphorylate AZT. The intracellular AZTMP peaked at 4 hr in trophoblasts and decreased subsequently, even in the presence of a constant extracellular AZT concentration (Fig. 1), whereas in Hofbauer cells AZTMP concentration peaked at 48 hr, suggesting that the balance of the anabolic and catabolic activities in these cells may differ. We also noted slightly lower ratios of AZTMP/AZTTP and AZTMP/AZTDP in trophoblasts than in Hofbauer cells isolated from the same placenta (Fig. 3). The difference was more prominent with cells from another placenta exposed to a higher range of AZT concentrations (Fig. 3, B and D). However, due to interindividual variation, this phenomenon will require further verification. The difference in efficiency between trophoblasts and Hofbauer cells in converting AZTMP to AZTDP and AZTTP cannot be explained on the basis of the 3-fold larger cell volume of trophoblasts, as an increase in cell cytoplasm would proportionally increase the enzymes that catalyze the formation of all three species of phosphorylated AZT. This event will result in constant ratios (AZTMP/AZTTP and AZTMP/AZTDP) for both cells.

In addition, intracellular phosphorylated AZT concentrations in trophoblasts and Hofbauer cells were 50- to 100-fold lower than the concentrations found in either human lymphocytic cell lines [20–23] or activated lymphocytes [24–28]. However, these phosphorylated AZT concentrations were comparable to those of resting peripheral blood mononuclear cells [27], suggesting a less active metabolism for AZT in both types of placental cells. As previously noted, phosphorylated metabolites in trophoblasts peaked at 4 hr and declined subsequently, a phenomenon previously reported for lymphocytic cell lines, such as ATH8, Molt/4F, MT-4 [29], CCRE-CEM [22], and peripheral blood mononuclear cells [24]. The exact mechanism of the

decline in intracellular AZT metabolites in the presence of constant extracellular AZT is not clear at present.

Although it is not known whether phosphorylated AZT metabolites may be toxic to human placental cells, it is possible that AZTTP, which interferes with cellular DNA synthesis, may affect placental growth, specifically of the trophoblasts. Previously, we reported that AZT inhibits hormone secretion and DNA synthesis in human placental trophoblasts in a concentration-dependent manner [18]. In the present work, we observed an AZT concentration-dependent inhibitory effect on cellular DNA synthesis that correlated with an increase in intracellular AZTTP concentration. This is consistent with the notion of chain termination [13, 17] of DNA synthesis in the trophoblasts by AZTTP (Fig. 6).

In addition to the effect on DNA synthesis, AZT also inhibits progesterone synthesis in trophoblasts [18]. The effect of AZT on placental cell progesterone synthesis may relate to its inhibitory effect on mitochondrial DNA synthesis [30, 31]. The mitochondrion is the major organelle involved in the conversion of cholesterol to progesterone in trophoblasts. AZTTP inhibits human mitochondrial DNA polymerase γ, an enzyme essential for mitochondrial DNA synthesis [16]. Our correlation studies (Fig. 6) indicate that the IC_{50} of AZTTP is less than 1 μM for inhibiting trophoblast progesterone production, a concentration produced by therapeutic doses of AZT.

Furman *et al.* [11] reported that AZTTP can compete with dTTP for HIV reverse transcriptase [inhibition constant (K_i) = 0.04 μM]. Under the condition of 5 μM extracellular AZT (AZT plasma concentrations of 5–10 μM are achieved in the clinic), the intracellular AZTTP concentration is estimated to be about 7.5-fold higher than that required to inhibit HIV reverse transcriptase (Fig. 2). After removal of 10 μM AZT from the medium, intracellular AZTTP concentration was well above the K_i value (0.04 μM) for at least 4 hr (Fig. 5). The AZTTP decay half-lives in trophoblasts and Hofbauer cells (4.6 and 5.0 hr, respectively) were slightly longer than those reported in leukemic T cell lines ($T_{1/2\beta}$ = 3–4 hr) [23, 32]. These data indicate that the presently used dosing regimens of AZT should be adequate to produce inhibitory concentrations of AZTTP in both trophoblasts and Hofbauer cells *in vivo*.

We observed a substantial release of AZTMP into the medium of the trophoblasts and Hofbauer cells, a phenomenon also reported for other human cell lines [21, 22, 24]. Fridland *et al.* [22] have proposed that an active transport system may be involved in the excretion mechanism of AZTMP. However, comparison between extracellular and intracellular concentrations of AZTMP indicates that AZTMP levels in the medium are at least 50-fold lower than those levels of intracellular concentration for both trophoblasts and Hofbauer cells (Fig. 4). Therefore, diffusion across the cell membrane is likely to be the key mechanism for the presence of AZTMP in the extracellular medium. Although monophosphorylated nucleosides are considered impermeable to the cell membrane, a higher lipophilicity of AZTMP (because of the azido group) may permit the intracellular AZTMP to diffuse across the membrane.

In summary, the data presented here provide important information regarding the phosphorylation of AZT in trophoblasts and Hofbauer cells, both of which are thought to play an important role in maternal–fetal HIV transmission. Assuming that the decay kinetics of intracellular AZTTP observed in these cells *in vitro* apply to the *in vivo* situation, the present AZT dosing regimens used in the clinic should provide intracellular AZTTP concentrations in trophoblasts and Hofbauer cells that are inhibitory to HIV replication.

Note added in proof—The results of the ACTG 076 trial were announced after preparing this manuscript. When women were administered AZT or placebo beginning at 14–36 weeks of gestation, AZT was found to significantly reduce transmission (by 68%) (Thomas E, Jr, AZT is found to curb HIV's fetal infection. *The Wall Street Journal*, Feb. 22, 1994, B8.) These data provide additional impetus to the study of mechanisms by which AZT produces this effect. Inter-individual differences in intracellular metabolism may be a contributory factor in individuals where AZT failed to prevent maternal–fetal HIV transmision.

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