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Research Paper

Characterization of the Mechanism of Zidovudine Uptake by Rat Conditionally Immortalized Syncytiotrophoblast Cell Line TR-TBT

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Purpose. To characterize the uptake mechanism of zidovudine (AZT), a nucleoside reverse transcriptase inhibitor, in syncytiotrophoblast cells using the TR-TBT 18d-1 cell line previously established by our group.
Materials and Methods. The effects of several transporter inhibitors on the initial and steady-state apical uptake of AZT by TR-TBT 18d-1 were characterized, in order to identify the transporter(s) involved.
Results. Initial uptake of AZT was sodium-independent and saturable; the K_m value was about 16 μM. Nitrobenzylthioinosine (NBMPR), probenecid and cimetidine each had little effect on the saturable AZT uptake, indicating that well characterized transporters, such as organic anion transporters (OATs and OATPs), organic cation transporters (OCTs) and equilibrative nucleoside transporters (ENTs), are not involved. However, thymidine and 2′-deoxyuridine strongly inhibited AZT uptake. These results suggest that an unidentified nucleoside uptake transporter is responsible for the uptake of AZT. Cyclosporin A, Ko143 and probenecid had little effect on AZT accumulation by TR-TBT 18d-1 cells, suggesting that transporter-mediated efflux of AZT is not substantial.
Conclusion. Our results indicate that saturable AZT uptake into TR-TBT 18d-1 is mediated by a so-far-unidentified transporter.

KEY WORDS: AZT; blood–placenta barrier; syncytiotrophoblast; transporter; TR-TBT.

INTRODUCTION

The placenta regulates transport of nutrients and exchange of gases between maternal and fetal blood, and the blood-placenta barrier (BPB), which is composed of syncytiotrophoblast cells, has a key role in these functions. Histologically, the placenta exhibits large species differences; for example, in rats, syncytiotrophoblast cells form a double layer (syncytiotrophoblast I, maternal side layer; syncytiotrophoblast II, fetal side layer), whereas the human placenta has a single layer of syncytiotrophoblast cells. Much research has been focused on the human placenta, since the tissue is relatively freely available compared with other organs. Nevertheless, it has been reported that transport functions in the BPB are similar in human and rat placenta (1). Therefore, we established conditionally immortalized rat syncytiotrophoblast cell lines, TR-TBTs (2) as an *in vitro* BPB model. Our studies indicated that TR-TBT 18d-1 best reflected the characteristics of syncytiotrophoblast I (3), so we adopted TR-TBT 18d-1 as a model cell line for the present study.

Zidovudine (3′-azido-3′-deoxythymidine, AZT) is a nucleoside reverse transcriptase inhibitor, which is used for the treatment of patients infected with human immunodeficiency virus (HIV). In pregnant women, AZT is used for prevention of mother-to-infant transmission of HIV. AZT is not teratogenic, at least in rats and rabbits, at doses up to 500 mg kg^{-1} day^{-1}, and no pattern of birth defects has been detected in humans (4). Throughout pregnancy, the plasma concentration of AZT in the mother is similar to that in the fetus (5). Therefore, AZT appears to be transported across the placenta, and the concentrations in the mother's blood and the fetal blood may be in equilibrium.

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ABBREVIATIONS: AZT, 3′-azido-3′-deoxythymidine; BCRP, breast cancer resistance protein; CNT, concentrative nucleoside transporter; DHEAS, dehydroepiandrosterone sulfate; ENT, equilibrative nucleoside transporter; MRP, multidrug resistance protein; NMBPR, nitrobenzylthioinosine; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; PAH, -aminohippuric acid; P-gp, p-glycoprotein.

Nucleosides are transported across the placenta via nucleoside transporters, such as concentrative nucleoside transporters (CNTs, SLC28) and equilibrative nucleoside transporters (ENTs, SLC29). We recently showed that uridine and adenosine are taken up via ENTs in TR-TBT cells (6). Several authors, however, have concluded that AZT, a nucleoside analog, is transferred to the placenta by simple diffusion (7–9). On the other hand, AZT is a substrate of several transporters, including CNTs (h/rCNT1 and hCNT3) (10,11), h/rENT2 (12), OATs (h/rOAT1, h/rOAT2, h/rOAT3 and hOAT4) (13–16), rOCT1 (17) and OAT-Ks (OAT-K1 and OAT-K2)(18). AZT is also a substrate of efflux transporters, such as P-gp (19), BCRP (20,21) and MRP4 (22). At the blood-brain barrier, AZT is excreted from the brain into the blood via a probenecid-sensitive efflux transporter (23). These results suggest that some transporter(s) may be involved in regulating the tissue distribution of AZT. Several transporters that are able to transport AZT are expressed in human placenta and in TR-TBT 18d-1. For example, CNT3, ENT2, OAT3, OCT1, OAT-K2, MDR1a, MRP4 and BCRP are expressed in TR-TBT 18d-1, at least at the mRNA level (3). P-gp (MDR1) and BCRP have been detected immunohistochemically in human placental trophoblast (24–26). Human placenta expresses mRNAs of CNT1, CNT3, ENT2, OAT2, OAT4, OCT1, MDR1, MRP4 and BCRP, which can transport AZT, to various extents (27). Accordingly, it remains possible that several transporters are involved in the BPB transport of AZT.

In the present study, we aimed to characterize the uptake mechanism of AZT in syncytiotrophoblast cells, using TR-TBT 18d-1 as a model. It is important to note that even if transplacental transport clearance is apparently similar to the clearance by simple diffusion, some transporter may still involved in the transport process across the apical or basolateral membrane.

MATERIALS AND METHODS

Chemicals

[^{3}H]AZT (12.7 Ci/mmol) was purchased from Moravek Biochemicals, Inc. (Brea, CA). Ko143 was synthesized as described previously (28). All other chemicals were commercial products of analytical grade.

Cell Culture

TR-TBT 18d-1 were cultured in Dulbecco's modified Eagle's medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 10% fetal bovine serum (SAFC Biosciences, Lenexa, KS), 100 U/ml benzylpenicillin, 100 μg/ml streptomycin, 2 mM L-glutamine (Invitrogen, San Diego, CA) on 100-mm culture dishes (Corning Life Sciences, Lowell, MA) in a humidified incubator at 33°C under an atmosphere of 5% CO_2 in air. For the uptake study, TR-TBT 18d-1 were seeded on four-well plates (Nalge Nunc International, Naperville, IL) coated with porcine skin collagen type I (Nitta Gelatin, Osaka, Japan) at a density of 1×10^5 cells/well. After incubation for 3 days at 37°C, the cells were cultured at 37°C for a further 4 days. The culture medium was changed every 2 days for 7 days.

Uptake Study

TR-TBT 18d-1 were washed twice with transport medium containing 122 mM NaCl, 25 mM $NaHCO_3$, 3 mM KCl, 1.4 mM $CaCl_2$, 1 mM $MgSO_4$, 0.4 mM K_2HPO_4, 10 mM D-glucose, and 10 mM Hepes adjusted to pH 7.4 with Tris. Sodium-free transport medium was prepared by replacing 122 mM NaCl and 25 mM $NaHCO_3$ with 122 mM *N*-methyl-D-glucamine and 25 mM $KHCO_3$. After a 10 min preincubation in the above medium, the transport medium was replaced with drug solution containing [^{3}H]AZT (143 nM, 18.5 kBq/ml) to start the uptake reaction. The reaction was terminated by addition of ice-cold transport medium and the cells were washed twice in the same medium. The cells were solubilized by incubation overnight in 500 μl of 0.1 M NaOH/1% Triton-X solution at 37°C. Next, 400 μl of the cell lysate was mixed with 3 ml of scintillation cocktail (Clearsol-I, Nacalai Tesque, Kyoto, Japan), and radioactivity was measured with a liquid scintillation counter (TRI-CARB 317TR/SL, Packard Instrument Company, Meriden, CT). Cellular protein was quantified using a protein assay kit (Pierce, Rockford, IL) with bovine serum albumin as a standard.

Data Analysis

Kinetic parameters for [^{3}H]AZT uptake by TR-TBT 18d-1 were obtained by nonlinear least-squares regression analysis based on the Michelis-Menten equation:

$$V = \frac{V_{max} \times S}{K_m + S} + k_{ns} \times S$$

where V, V_{max}, K_m, S and k_{ns} represents initial uptake velocity, maximum uptake velocity, Michaelis constant, substrate concentration and non-saturable uptake clearance, respectively.

Statistical analyses were performed by one-way ANOVA with Dunnett's post-hoc test.

RESULTS

Time Course of [^{3}H]AZT Uptake by TR-TBT 18d-1

To characterize the [^{3}H]AZT uptake by TR-TBT 18d-1, we first measured [^{3}H]AZT uptake for 30 min. [^{3}H]AZT uptake increased in a time-dependent manner, and initial uptake was evaluated at 5 min in the following studies (Fig. 1). [^{3}H]AZT uptake was markedly inhibited by the addition of 20 mM unlabeled AZT (Fig. 1). This saturable transport component indicated the involvement of some kind of transporter. [^{3}H]AZT uptake by TR-TBT 18d-1 under sodium-free conditions was 64.3±2.1 (μl/mg protein per 5 min) and was not significantly different from the control in the presence of sodium. Therefore, the saturable transport appears to be mediated by a sodium-independent transporter(s).

Kinetic Analysis of [^{3}H]AZT Uptake by TR-TBT 18d-1

To investigate the transporter responsible for [^{3}H]AZT uptake by TR-TBT 18d-1, we analyzed the concentration

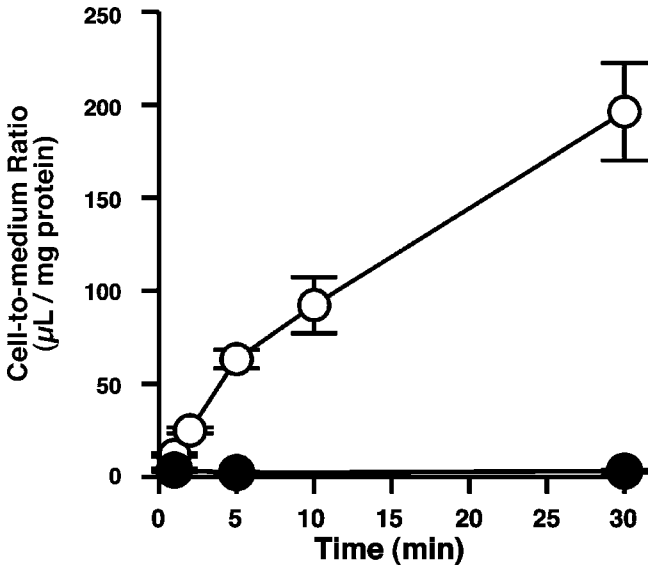

Fig. 1. Time course of [^{3}H]AZT uptake by TR-TBT 18d-1 [^{3}H]AZT (143 nM) uptake was measured at 37°C in the absence (*empty circle*) and presence (*filled circle*) of an excess (20 mM) of unlabeled AZT. Cellular uptake of [^{3}H]AZT was represented by cell-to-medium ratio, obtained by dividing the uptake amount by the [^{3}H]AZT concentration in the transport medium. Each point represents the mean±SEM of four determinations.

dependence of [^{3}H]AZT uptake. [^{3}H]AZT uptake was well fitted to the Michaelis–Menten equation, and an Eadie–Hofstee plot was linear (Fig. 2). The estimated kinetic parameters (K_m, V_{max} and k_{ns}) were 16.0±2.2 (μM), 980±68 (pmol/mg protein per 5 min) and 1.51±0.19 (μL/mg protein per 5 min), respectively. Saturable transport (V_{max}/K_m) accounted for approximately 98% of the total uptake clearance ($V_{max}/K_m + K_{ns}$) when the AZT concentration was much less than the K_m value, i.e., when K_m+S is approximately equal to K_m (actually up to about 640 nM, which is close to the unbound AZT concentration in the clinical situation).

Characterization of [^{3}H]AZT Uptake by TR-TBT 18d-1

To identify the sodium-independent transporter responsible for [^{3}H]AZT uptake, we investigated the effects of several inhibitors of OATs, OCTs and ENTs, which are candidate transporters for [^{3}H]AZT uptake by TR-TBT 18d-1. These inhibitors had little effect on [^{3}H]AZT uptake, indicating that the contributions of OATs, OCTs and ENTs to [^{3}H]AZT uptake by TR-TBT 18d-1 were negligible (Fig. 3A). Unexpectedly, DHEAS significantly increased the initial uptake of [^{3}H]AZT (Fig. 3A). Since DHEAS is a substrate of the OATP family, we next examined the effect of other OATP substrates. Interestingly estrone-3-sulfate showed slight but significant enhancement of AZT uptake (Fig. 3B). Two other OATP substrates, estradiol-17-ß-glucuronide and taurocholic acid, had little effect on AZT uptake (Fig. 3B). The organic cation tetraethylammonium (TEA), used as a negative control, also had no effect (Fig. 3B). Consequently, none of the OATP substrates used in the current study inhibited AZT uptake.

Since the involvement of well-characterized transporters seemed to be negligible, it seemed possible that some unidentified transporter(s) is involved in [^{3}H]AZT uptake by TR-TBT 18d-1. To assess the uptake mechanism of [^{3}H]AZT, structural analogs were used as inhibitors. Thymidine and 2′-

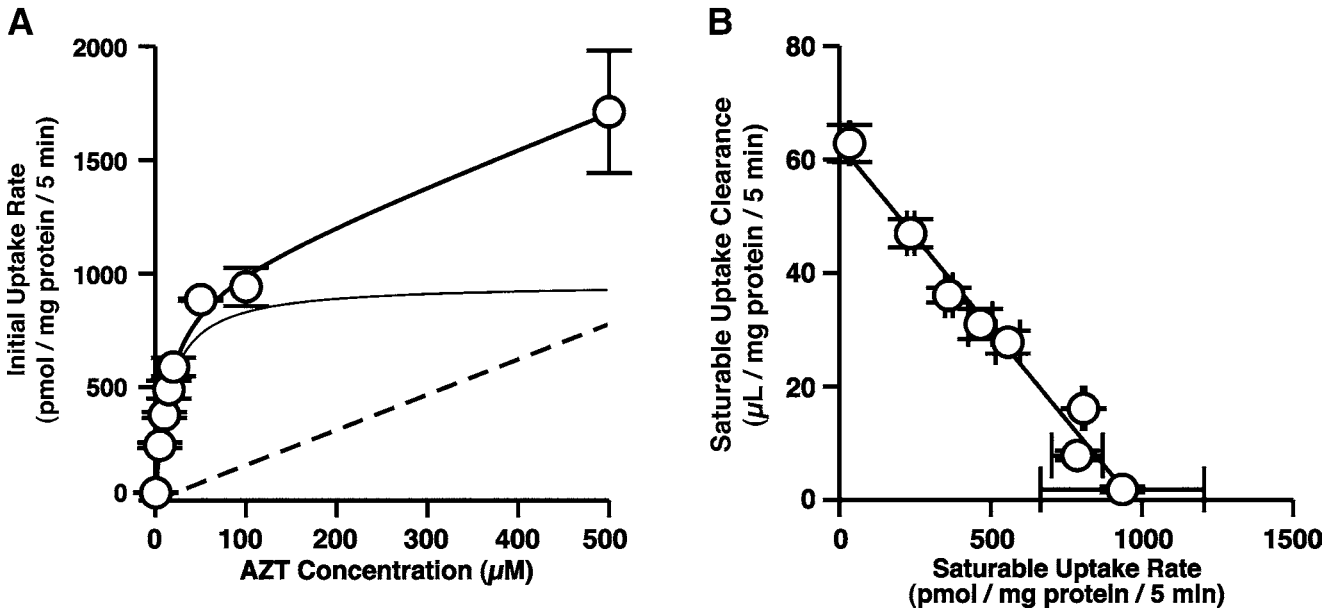

Fig. 2. Kinetic analysis of [^{3}H]AZT uptake by TR-TBT 18d-1 [^{3}H]AZT (143 nM) uptake was measured at 37°C for 5 min in the presence of 0.5–500 μM unlabeled AZT. **A** Kinetic analysis was performed by use of the Michaelis–Menten equation as described in "MATERIALS AND METHODS" and the fitting curve is shown as a bold line. Saturable and non-saturable components are shown as thin and dotted lines, respectively. **B** An Eadie-Hoffstee plot of the saturable component is shown. Each point represents the mean±SEM of four determinations.

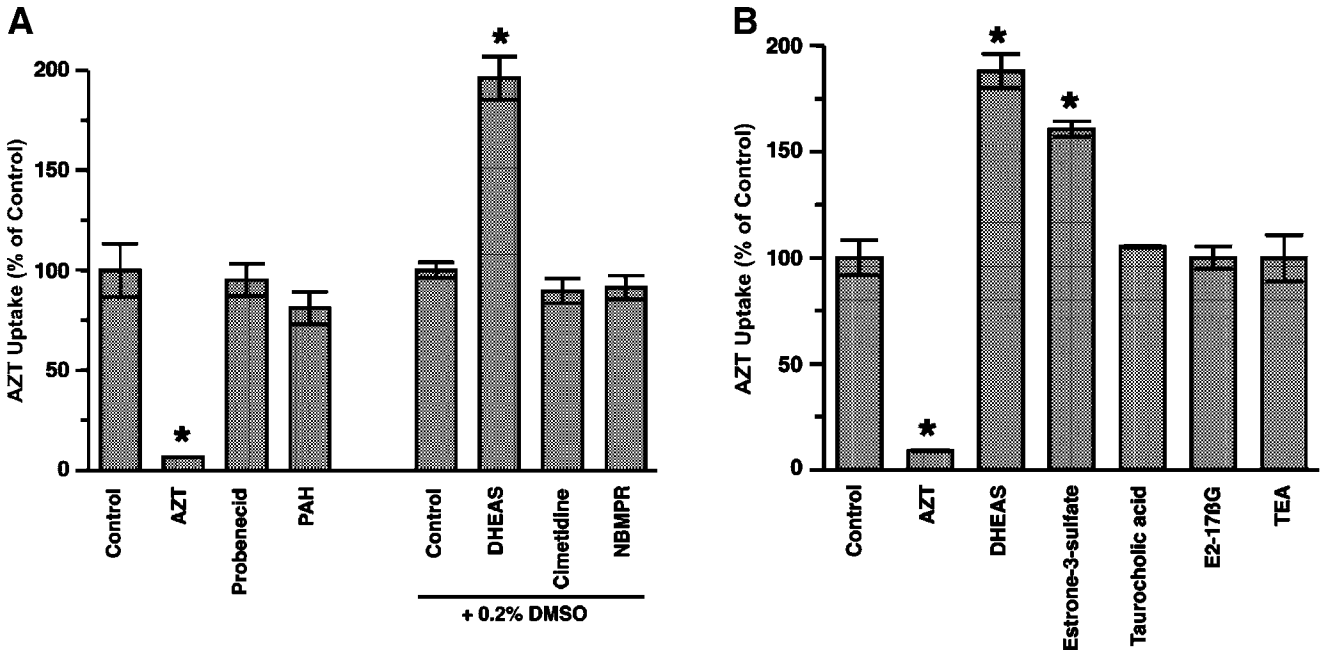

Fig. 3. Effect of several inhibitors on [^{3}H]AZT uptake by TR-TBT 18d-1 [^{3}H]AZT (143 nM) uptake was measured at 37°C for 5 min in the presence of designated inhibitors. AZT (1 mM) was used as a control. **A** Probenecid, PAH, and DHEAS were used as inhibitors of organic anion transporters at 2 mM. Cimetidine was used as an organic cation transporter inhibitor at 2 mM. NBMPR was used as an inhibitor of equilibrative nucleotide transporters at 0.1 mM. DMSO (0.2%) was added to dissolve DHEAS, cimetidine and NBMPR. **B** The inhibitory effects of estrone-3-sulfate (1 mM), estradiol-17-ß-glucuronide (E2-17ßG, 0.1 mM), taurocholic acid (1 mM) and tetraethylammonium (TEA, 1 mM) were measured. Each point represents the mean±SEM of three to four determinations. *Asterisk* Significant difference from the control ($p<0.05$).

deoxyuridine inhibited [^{3}H]AZT uptake as strongly as AZT itself (Fig. 4). All the other structural analogs used in the current study also significantly inhibited [^{3}H]AZT uptake (Fig. 4). These results suggested that a transporter(s) that strongly recognizes nucleosides was involved in [^{3}H]AZT uptake by TR-TBT 18d-1.

Effect of Concurrently Administered Drugs on [^{3}H]AZT Uptake

AZT is not always administered alone, but is often used with concurrent drugs. We therefore investigated possible interactions in AZT transport in syncytiotrophoblast cells. Lamivudine showed little inhibition, even though it is a nucleoside reverse transcriptase inhibitor, like AZT and stavudine (Fig. 5). Ganciclovir slightly decreased [^{3}H]AZT uptake at a high concentration (1,000 μM), but a low concentration (50 μM) showed no significant inhibition. Pentamidine and azithromycin had little effect on [^{3}H]AZT uptake.

Involvement of Efflux Transporters in [^{3}H]AZT Uptake by TR-TBT 18d-1

To understand the dynamics of [^{3}H]AZT uptake by TR-TBT 18d-1, we investigated the possible involvement of efflux transporters. For this purpose, we performed inhibition studies with several efflux transporter inhibitors to determine the effects on steady-state [^{3}H]AZT accumulation in TR-TBT 18d-1. Since [^{3}H]AZT uptake showed little further increase after 30 min (data not shown), we adopted 60 min as a steady-state condition. The P-gp and MRP family inhibitor, cyclosporin A, and BCRP inhibitor, Ko143, did not increase [^{3}H]AZT uptake by TR-TBT 18d-1 (Fig. 6). Probenecid, a non-specific organic anion transport system inhibitor, also had no effect on [^{3}H]AZT uptake. These results imply that the role, if any, of efflux transporters is minor.

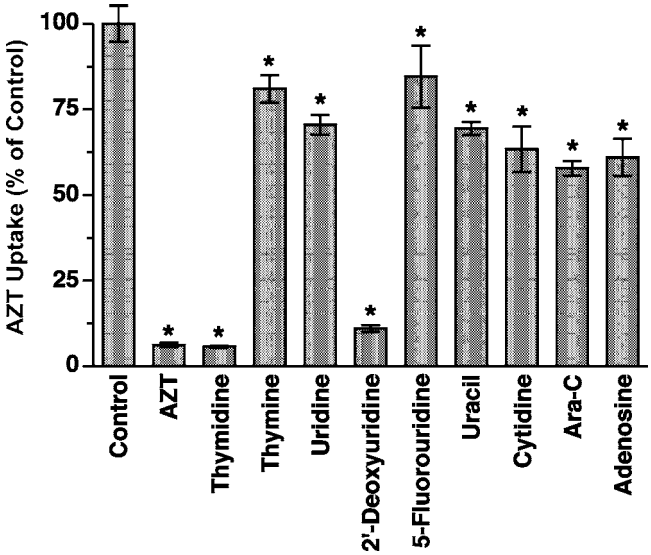

Fig. 4. Inhibitory effect of nucleosides and nucleoside analogs on [^{3}H]AZT uptake by TR-TBT 18d-1 [^{3}H]AZT (143 nM) uptake was measured at 37°C for 5 min in the presence of 1 mM inhibitor. Each point represents the mean±SEM of three to four determinations. *Asterisk* Significant difference from the control ($p<0.05$).

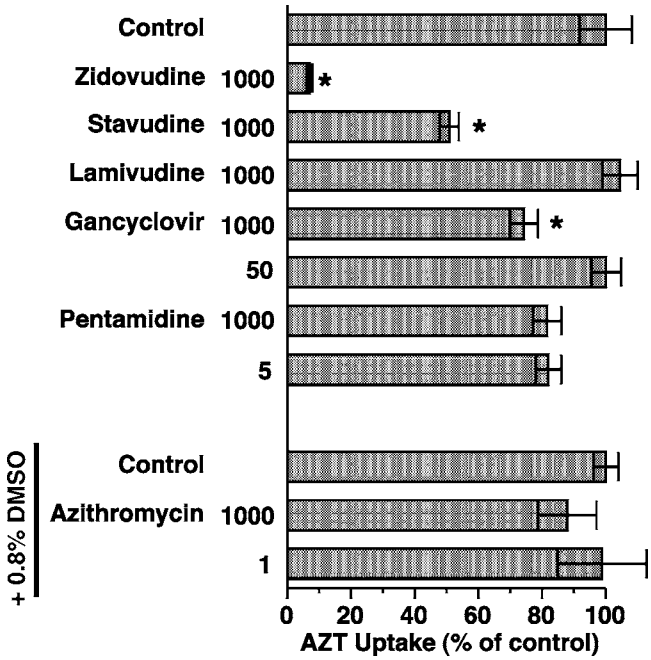

Fig. 5. Inhibitory effect of possible concurrently administered drugs on [^{3}H]AZT uptake by TR-TBT 18d-1 [^{3}H]AZT (143 nM) uptake was measured at 37°C for 5 min in the presence of designated compounds (concentration, μM), which may be concomitantly administered in drug therapy. Each point represents the mean±SEM of three to four determinations. *Asterisk* Significant difference from the control ($p<0.05$).

gated (Fig. 3). PAH and probenecid are well-known inhibitors of OAT1 and organic anion transport system(s), respectively, but they had no significant inhibitory effect, indicating that OAT family members are not involved in AZT uptake by TR-TBT 18d-1, despite the apparent similarity of K_m values. Cimetidine and NBMPR were used as inhibitors of OCTs (organic cation transporters) and ENTs (equilibrative nucleoside transporters) (35,36), respectively, but were ineffective, indicating that the contributions of OCT1, ENT1 and ENT2 to AZT uptake are negligible. DHEAS is a substrate of several members of the OATP family and effectively inhibits the activity of OAT-Ks (18). Interestingly, DHEAS facilitated the initial uptake of AZT (Fig. 3). DHEAS itself is taken up by TR-TBT 18d-1 via a transporter(s) which has not identified yet (3), so we examined the effect of some representative substrates of OATP family members on AZT uptake by TR-TBT 18d-1. Interestingly, estrone-3-sulfate showed a slight but significant enhancement of AZT uptake, although the enhancement was less than that by DHEAS (Fig. 3B). The lack of an enhancing effect of taurocholic acid and estradiol-17-ß-glucuronide suggests that the effects of DHEAS and estrone-3-sulfate may be specific effects on the unidentified transporter, rather than stimulatory effects on known OATP family members, although we can not completely exclude the possible involvement of the OATP family, since several reports have shown that OATP substrates can enhance the transport of another substrate (37,38). Further studies are required to clarify the mechanism of the facilitated uptake of AZT in the presence of DHEAS

DISCUSSION

Our results indicate that AZT uptake across the apical membrane of the rat conditionally immortalized syncytiotrophoblast cell line TR-TBT 18d-1 is mediated by a transporter (s). The TR-TBT cell line was established by our group, and has been confirmed to express trophoblast markers. We isolated four clones (18d-1 to -4) of TR-TBT from a transgenic rat harboring the temperature-sensitive simian virus 40 large T-antigen gene at 18 days of gestation. Among the 4 clones, TR-TBT 18d-1 used in the present study shows polarization and apical localization of GLUT1 and GLUT3, indicating that it has the histological characteristics of syncytiotrophoblast I (2). Moreover, TR-TBT 18d-1 expressed a number of transporter mRNAs, and also showed transporter-mediated uptake activity towards DHEAS, an essential substance for fetal development (3). Therefore, TR-TBT 18d-1 was expected to be a suitable tool for the study of transport activities in the BPB.

As noted in the introduction, AZT is a substrate of CNTs (CNT1 and CNT3), ENT2, OATs (OAT1, OAT2, OAT3 and OAT4), and OCT1 (10–17). The CNTs are sodium-dependent and ENT2, OATs and OCT1 are sodium-independent transporters (29–34). TR-TBT 18d-1 express mRNAs of CNT2 and CNT3, although not CNT1 (2). [^{3}H]AZT uptake did not show sodium dependency (see "RESULTS"), indicating that the contribution of CNTs is minimal.

Kinetic analysis revealed that AZT uptake by TR-TBT 18D-1 cells was predominantly saturable (Fig. 2). The K_m value for AZT uptake was approximately 16 μM, which is fairly close to those of OATs (26–145 μM) (13–16). Therefore, to identify the transporter(s) responsible for AZT uptake by TR-TBT 18d-1, the effects of several inhibitor(s) were investi-

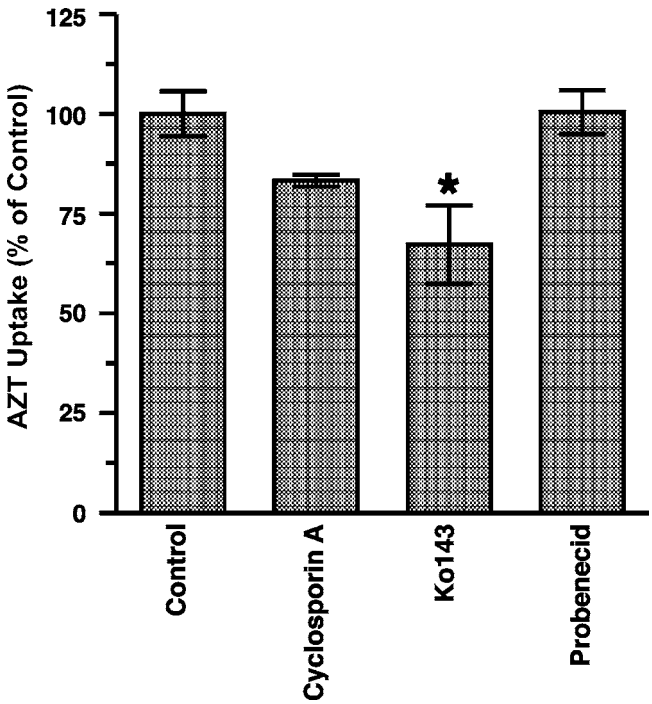

Fig. 6. Effect of inhibitors of efflux transporters on [^{3}H]AZT uptake by TR-TBT 18d-1 [^{3}H]AZT (143 nM) uptake was measured at 37°C for 60 min in the presence of designated inhibitors. Cyclosporin A, Ko143 and probenecid were used at the concentrations of 10 μM, 10 μM and 2 mM, respectively. Each point represents the mean± SEM of three to four determinations. *Asterisk* Significant difference from the control ($p<0.05$).

and estrone-3-sulfate. Overall, these results indicated that an unknown transporter is responsible for the AZT uptake.

Some transporters are known to be up-regulated in TR-TBT 18d-1 (2,3), so we can not rule out the possibility that an up-regulated transport protein(s) is involved in AZT uptake in this cell line. Further analysis is required to show whether a transporter not specifically expressed in TR-TBT 18d-1 is involved in the AZT uptake, and if so, to clarify its contribution to AZT uptake by syncytiotrophoblasts under physiological conditions.

We also performed an inhibition study using structural analogs, such as nucleosides (Fig. 4). Thymidine inhibited AZT uptake as potently as AZT itself, and thymine was slightly inhibitory. Similarly, 2′-deoxyuridine strongly inhibited AZT uptake by TR-TBT 18d-1, being more potent than uridine, 5-fluorouridine or uracil (Fig. 4). Cytidine, Ara-C and adenosine showed slight inhibition of AZT uptake (Fig. 4). These results indicate that the transporter responsible for AZT uptake recognizes 2′-deoxynucleosides more effectively than nucleosides and nucleobases. A transporter specific for 2′-deoxynucleosides has not yet been isolated, although ENT2 is known to transport 3′-deoxynucleosides. Further investigation is needed to characterize the transporter responsible for AZT uptake.

Anti-HIV agents are often administered concomitantly with other drugs. Considering the species difference between humans and rats and the *in vitro–in vivo* relationship between TR-TBT 18d-1 and intact syncytiotrophoblasts, it is difficult to estimate the likelihood of drug-drug interaction under physiological conditions based on the results of the present study. Therefore, for the present purpose, we tentatively assume that TR-TBT 18d-1 have similar characteristics to human syncytiotrophoblasts. Lamivudine, pentamidine and azithromycin had little effect on AZT uptake (Fig. 5), and so may not inhibit AZT uptake under physiological conditions. Although ganciclovir inhibited AZT uptake at 1,000 μM, a physiologically relevant concentration (50 μM) was not markedly inhibitory (Fig. 5), suggesting that ganciclovir might have little effect on AZT uptake under physiological conditions. To support these speculations, it will be necessary to compare drug transport characteristics in human syncytiotrophoblasts and TR-TBT 18d-1.

To understand the dynamics of AZT uptake by TR-TBT 18d-1, we investigated the possible involvement of efflux transporters. The P-gp and MRP family inhibitor, cyclosporin A, and the BCRP inhibitor, Ko143, hardly increased [^{3}H] AZT uptake by TR-TBT 18d-1 cells (Fig. 6) under conditions where they inhibited efflux transport of AZT in P-gp-expressing LLC-PK1 cells and Bcrp1-transfected MDCKII cells, respectively (21,39). BCRP expression in TR-TBT 18d-1 may be too low to influence AZT accumulation, since it was recently reported that Ko143 can inhibit BCRP-mediated AZT efflux (3,21). Probenecid, a multi-specific organic anion transport system inhibitor, also did not change [^{3}H]AZT uptake, implying that MRP family efflux transporters are unlikely to be involved in AZT uptake by TR-TBT 18d-1.

Although we observed saturable uptake of AZT, other authors have reported that AZT penetrates the BPB by simple diffusion (7–9). This apparent conflict could be explained in several ways. For example, (1) AZT is taken up by a transporter(s) across the apical membrane of syncytiotrophoblast, but transferred to the fetal side across the basal membrane by simple diffusion, (2) the free drug concentration was not sufficiently high for competitive inhibition in the *in vivo* situation due to serum protein binding.

In summary, the present study indicated that AZT uptake is primarily transporter-mediated in TR-TBT 18d-1. Well-characterized transporters were not involved, suggesting that an unidentified transporter is mainly responsible for AZT uptake by TR-TBT 18d-1. These findings may be important for anti-HIV therapy of pregnant women, and should be useful in the development of novel nucleoside reverse transcriptase inhibitors.

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REFERENCES

1. K. Takata, and H. Hirano. Mechanism of glucose transport across the human and rat placental barrier: a review. *Microsc. Res. Tech.* **38**:145–152 (1997).
2. T. Kitano, H. Iizasa, T. Terasaki, T. Asashima, N. Matsunaga, N. Utoguchi, Y. Watanabe, M. Obinata, M. Ueda, and E. Nakashima. Polarized glucose transporters and mRNA expression properties in newly developed rat syncytiotrophoblast cell lines, TR-TBTs. *J. Cell Physiol.* **193**:208–218 (2002).
3. T. Kitano, H. Iizasa, I. W. Hwang, Y. Hirose, T. Morita, T. Maeda, and E. Nakashima. Conditionally immortalized syncytiotrophoblast cell lines as new tools for study of the blood-placenta barrier. *Biol. Pharm. Bull.* **27**:753–759 (2004).
4. G. G. Briggs, R. K. Freeman, and S. J. Yaffe. *Drugs in pregnancy and lactation*. Lippincott Williams and Wilkins, Philadelphia, (2005).
5. M. J. O'Sullivan, P. J. Boyer, G. B. Scott, W. P. Parks, S. Weller, M. R. Blum, J. Balsley, and Y. J. Bryson. The pharmacokinetics and safety of zidovudine in the third trimester of pregnancy for women infected with human immunodeficiency virus and their infants: phase I acquired immunodeficiency syndrome clinical trials group study (protocol 082). Zidovudine Collaborative Working Group. *Am. J. Obstet. Gynecol.* **168**:1510–1516 (1993).
6. T. Chishu, Y. Sai, T. Nishimura, K. Sato, N. Kose, and E. Nakashima. Potential of various drugs to inhibit nucleoside uptake in rat syncytiotrophoblast cell line, TR-TBT 18d-1. *Placenta*, in press (2008).
7. L. Liebes, S. Mendoza, D. Wilson, and J. Dancis. Transfer of zidovudine (AZT) by human placenta. *J. Infect. Dis.* **161**:203–207 (1990).
8. S. Schenker, R. F. Johnson, T. S. King, R. S. Schenken, and G. I. Henderson. Azidothymidine (zidovudine) transport by the human placenta. *Am. J. Med. Sci.* **299**:16–20 (1990).
9. J. Dancis, J. Lee, S. Mendoza, and L. Liebes. Nucleoside transport by perfused human placenta. *Placenta*. **14**:547–554 (1993).

10. S. Y. Yao, C. E. Cass, and J. D. Young. Transport of the antiviral nucleoside analogs 3′-azido-3′-deoxythymidine and 2′,3′-dideoxycytidine by a recombinant nucleoside transporter (rCNT) expressed in Xenopus laevis oocytes. *Mol. Pharmacol.* **50**:388–393 (1996).
11. M. W. Ritzel, A. M. Ng, S. Y. Yao, K. Graham, S. K. Loewen, K. M. Smith, R. J. Hyde, E. Karpinski, C. E. Cass, S. A. Baldwin, and J. D. Young. Recent molecular advances in studies of the concentrative Na+-dependent nucleoside transporter (CNT) family: identification and characterization of novel human and mouse proteins (hCNT3 and mCNT3) broadly selective for purine and pyrimidine nucleosides (system cib). *Mol. Membr. Biol.* **18**:65–72 (2001).
12. S. Y. Yao, A. M. Ng, M. Sundaram, C. E. Cass, S. A. Baldwin, and J. D. Young. Transport of antiviral 3′-deoxy-nucleoside drugs by recombinant human and rat equilibrative, nitrobenzylthioinosine (NBMPR)-insensitive (ENT2) nucleoside transporter proteins produced in Xenopus oocytes. *Mol. Membr. Biol.* **18**:161–167 (2001).
13. S. Wada, M. Tsuda, T. Sekine, S. H. Cha, M. Kimura, Y. Kanai, and H. Endou. Rat multispecific organic anion transporter 1 (rOAT1) transports zidovudine, acyclovir, and other antiviral nucleoside analogs. *J. Pharmacol. Exp. Ther.* **294**:844–849 (2000).
14. N. Morita, H. Kusuhara, T. Sekine, H. Endou, and Y. Sugiyama. Functional characterization of rat organic anion transporter 2 in LLC-PK1 cells. *J. Pharmacol. Exp. Ther.* **298**:1179–1184 (2001).
15. M. Takeda, S. Khamdang, S. Narikawa, H. Kimura, Y. Kobayashi, T. Yamamoto, S. H. Cha, T. Sekine, and H. Endou. Human organic anion transporters and human organic cation transporters mediate renal antiviral transport. *J. Pharmacol. Exp. Ther.* **300**:918–924 (2002).
16. M. Hasegawa, H. Kusuhara, H. Endou, and Y. Sugiyama. Contribution of organic anion transporters to the renal uptake of anionic compounds and nucleoside derivatives in rat. *J. Pharmacol. Exp. Ther.* **305**:1087–1097 (2003).
17. R. Chen, and J. A. Nelson. Role of organic cation transporters in the renal secretion of nucleosides. *Biochem. Pharmacol.* **60**:215–219 (2000).
18. A. Takeuchi, S. Masuda, H. Saito, T. Abe, and K. Inui. Multispecific substrate recognition of kidney-specific organic anion transporters OAT-K1 and OAT-K2. *J. Pharmacol. Exp. Ther.* **299**:261–267 (2001).
19. G. Antonelli, O. Turriziani, M. Cianfriglia, E. Riva, G. Dong, A. Fattorossi, and F. Dianzani. Resistance of HIV-1 to AZT might also involve the cellular expression of multidrug resistance P-glycoprotein. *AIDS Res. Hum. Retroviruses.* **8**:1839–1844 (1992).
20. X. Wang, T. Nitanda, M. Shi, M. Okamoto, T. Furukawa, Y. Sugimoto, S. Akiyama, and M. Baba. Induction of cellular resistance to nucleoside reverse transcriptase inhibitors by the wild-type breast cancer resistance protein. *Biochem. Pharmacol.* **68**:1363–1370 (2004).
21. G. Pan, N. Giri, and W. F. Elmquist. Abcg2/Bcrp1 mediates the polarized transport of antiretroviral nucleosides abacavir and zidovudine. *Drug Metab. Dispos.* **35**:1165–1173 (2007).
22. J. D. Schuetz, M. C. Connelly, D. Sun, S. G. Paibir, P. M. Flynn, R. V. Srinivas, A. Kumar, and A. Fridland. MRP4: A previously unidentified factor in resistance to nucleoside-based antiviral drugs. *Nat. Med.* **5**:1048–1051 (1999).
23. K. Takasawa, T. Terasaki, H. Suzuki, and Y. Sugiyama. *In vivo* evidence for carrier-mediated efflux transport of 3′-azido-3′-deoxythymidine and 2′,3′-dideoxyinosine across the blood-brain barrier via a probenecid-sensitive transport system. *J. Pharmacol. Exp. Ther.* **281**:369–375 (1997).
24. C. Cordon-Cardo, J. P. O'Brien, D. Casals, L. Rittman-Grauer, J. L. Biedler, M. R. Melamed, and J. R. Bertino. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood–brain barrier sites. *Proc. Natl. Acad. Sci. U. S. A.* **86**:695–698 (1989).
25. M. Maliepaard, G. L. Scheffer, I. F. Faneyte, M. A. van Gastelen, A. C. Pijnenborg, A. H. Schinkel, M. J. van De Vijver, R. J. Scheper, and J. H. Schellens. Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. *Cancer Res.* **61**:3458–3464 (2001).
26. T. Litman, U. Jensen, A. Hansen, K. M. Covitz, Z. Zhan, P. Fetsch, A. Abati, P. R. Hansen, T. Horn, T. Skovsgaard, and S. E. Bates. Use of peptide antibodies to probe for the mitoxantrone resistance-associated protein MXR/BCRP/ABCP/ABCG2. *Biochim. Biophys. Acta.* **1565**:6–16 (2002).
27. M. Nishimura, and S. Naito. Tissue-specific mRNA expression profiles of human ATP-binding cassette and solute carrier transporter superfamilies. *Drug Metab. Pharmacokinet.* **20**:452–477 (2005).
28. T. Asashima, S. Hori, S. Ohtsuki, M. Tachikawa, M. Watanabe, C. Mukai, S. Kitagaki, N. Miyakoshi, and T. Terasaki. ATP-binding cassette transporter G2 mediates the efflux of phototoxins on the luminal membrane of retinal capillary endothelial cells. *Pharm. Res.* **23**:1235–1242 (2006).
29. J. Wang, S. F. Su, M. J. Dresser, M. E. Schaner, C. B. Washington, and K. M. Giacomini. Na(+)-dependent purine nucleoside transporter from human kidney: cloning and functional characterization. *Am. J. Physiol.* **273**:F1058–F1065 (1997).
30. S. A. Baldwin, P. R. Beal, S. Y. Yao, A. E. King, C. E. Cass, and J. D. Young. The equilibrative nucleoside transporter family, SLC29. *Pflugers Arch.* **447**:735–743 (2004).
31. T. Sekine, S. H. Cha, M. Tsuda, N. Apiwattanakul, N. Nakajima, Y. Kanai, and H. Endou. Identification of multispecific organic anion transporter 2 expressed predominantly in the liver. *FEBS Lett.* **429**:179–182 (1998).
32. T. Sekine, N. Watanabe, M. Hosoyamada, Y. Kanai, and H. Endou. Expression cloning and characterization of a novel multispecific organic anion transporter. *J. Biol. Chem.* **272**:18526–18529 (1997).
33. H. Kusuhara, T. Sekine, N. Utsunomiya-Tate, M. Tsuda, R. Kojima, S. H. Cha, Y. Sugiyama, Y. Kanai, and H. Endou. Molecular cloning and characterization of a new multispecific organic anion transporter from rat brain. *J. Biol. Chem.* **274**:13675–13680 (1999).
34. H. Koepsell, and H. Endou. The SLC22 drug transporter family. *Pflugers Arch.* **447**:666–676 (2004).
35. S. Y. Yao, A. M. Ng, W. R. Muzyka, M. Griffiths, C. E. Cass, S. A. Baldwin, and J. D. Young. Molecular cloning and functional characterization of nitrobenzylthioinosine (NBMPR)-sensitive (es) and NBMPR-insensitive (ei) equilibrative nucleoside transporter proteins (rENT1 and rENT2) from rat tissues. *J. Biol. Chem.* **272**:28423–28430 (1997).
36. Y. Urakami, M. Okuda, S. Masuda, H. Saito, and K. I. Inui. Functional characteristics and membrane localization of rat multispecific organic cation transporters, OCT1 and OCT2, mediating tubular secretion of cationic drugs. *J. Pharmacol. Exp. Ther.* **287**:800–805 (1998).
37. I. Tamai, T. Nozawa, M. Koshida, J. Nezu, Y. Sai, and A. Tsuji. Functional characterization of human organic anion transporting polypeptide B (OATP-B) in comparison with liver-specific OATP-C. *Pharm. Res.* **18**:1262–1269 (2001).
38. M. Grube, K. Kock, S. Karner, S. Reuther, C. A. Ritter, G. Jedlitschky, and H. K. Kroemer. Modification of OATP2B1-mediated transport by steroid hormones. *Mol. Pharmacol.* **70**:1735–1741 (2006).
39. S. Ekins, W. J. Crumb, R. D. Sarazan, J. H. Wikel, and S. A. Wrighton. Three-dimensional quantitative structure-activity relationship for inhibition of human ether-a-go-go-related gene potassium channel. *J. Pharmacol. Exp. Ther.* **301**:427–434 (2002).