Effect of Age on Distribution of Zidovudine (Azidothymidine) into the Cerebrospinal Fluid of *Macaca nemestrina*

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The brain tissue is an important target for anti-HIV drug therapy. Since the permeability of the blood–brain and blood–cerebrospinal fluid (CSF) barriers may differ between neonates and adults, we have determined the effect of age on the distribution of zidovudine (ZDV or azidothymidine) into the CSF in the macaque (*M. nemestrina*). Five newborn macaques were administered ZDV (iv bolus, 5 mg/kg) at various ages (2 days to 4 months). Both CSF (cisternal) and venous blood samples were obtained at approximately 60 and 90 min after drug administration. In another series of experiments, adult female macaques received ZDV as either an iv bolus (5 and 10 mg/kg) or an infusion for at least 12 hr. CSF (lumbar) and venous blood samples were obtained at approximately 60 and 90 min after iv bolus and at more than 12 hr after iv infusion. ZDV concentration in the CSF and the plasma samples was determined by high-performance liquid chromatography. The CSF/plasma concentration ratio of ZDV in the newborn and adult macaques, after iv bolus administration, was independent of time. In addition, no significant ($P > 0.05$) difference was observed in the pooled iv bolus ZDV CSF/plasma concentration ratio between the adult group (0.236 ± 0.058) and the newborns (0.213 ± 0.039). Moreover, the ZDV CSF/plasma concentration ratio in the adults and the newborns, after iv bolus administration, was found not to be significantly ($P > 0.05$) different from the ratio obtained at steady state in the adults (0.224 ± 0.094). These data indicate that the distribution of ZDV into the CSF in macaque neonates and adults is similar.

KEY WORDS: CSF; plasma; zidovudine; azidothymidine; infant; neonate; macaques; AIDS; CSF–blood barrier; biological transport.

INTRODUCTION

The human immunodeficiency virus (HIV) infects the central nervous system tissue, leading to dementia in adults and retardation of neurological development in infants (1,2). Zidovudine (ZDV or azidothymidine) is one of two approved drugs that are effective for the treatment of the neurological insult produced by HIV infection (3–5). Although the brain is an important target for drug therapy, the concentration of ZDV achieved in the brain is, for ethical reasons, not measurable. Instead, the concentration of ZDV achieved in the cerebrospinal fluid (CSF) is used as a surrogate, but not necessarily perfect, measure of the concentration of the drug in the extracellular fluid of the brain (6,7).

The steady-state concentration of ZDV in the CSF of children older than 14 months is a small fraction (0.24) of the corresponding concentration in the plasma (7). For ethical reasons, studies on the penetration of ZDV into the CSF in neonates have not been conducted. Since the permeability of the blood–brain and blood–CSF barriers is often markedly different in neonates compared with adults (8), ZDV may distribute into the brain and the CSF compartment more extensively in neonates compared with adults. To address this hypothesis, we have conducted age-related studies on the distribution of ZDV into the CSF in a representative animal model, the macaque (*M. nemestrina*). In addition, we have compared these findings with those obtained in adult macaques.

MATERIALS AND METHODS

Five neonate macaques were administered ZDV as an iv bolus (5 mg/kg) at various ages (2 days to 4 months) after birth as described previously (9). In addition to blood samples obtained for pharmacokinetic characterization, CSF and blood samples were obtained by cisternal and femoral vein puncture, respectively, approximately 60 and 90 min after drug administration. Similarly, in a study described previously (10), CSF samples were obtained from adult female pregnant macaques ($n = 5$) by lumbar puncture (lower thoracic region, under ketamine sedation, 5 mg/kg) approximately 60 and 90 min after iv bolus administration of ZDV (5 or 10 mg/kg). In a separate experiment, these pregnant females also received ZDV as an iv infusion at a rate sufficient to achieve a steady-state ZDV plasma concentration of 1 μg/mL (11). To ensure steady-state conditions, given that the plasma half-life of ZDV in the pregnant macaque is approximately 0.5 hr (10), a CSF and blood sample pair was obtained after at least 12 hr of infusion. Usually, the components of the CSF and blood sample pair were obtained within 5 min of each other, with an occasional sample pair being drawn 10 min apart. The concentrations of ZDV and its major metabolite, zidovudine glucoronide (ZDVG), in the CSF

Table I. ZDV CSF/Plasma Ratio in Adult and Infant Macaques After iv Bolus Administration

Age	Sampling time (min)	*n*	Mean ± SD	*P* value
Adult	60	4	0.216 ± 0.063	
Adult	90	4	0.256 ± 0.044	0.402
Infant	60	11[a]	0.199 ± 0.035	
Infant	90	11[a]	0.225 ± 0.044	0.076

[a] Only paired samples are included in this analysis.

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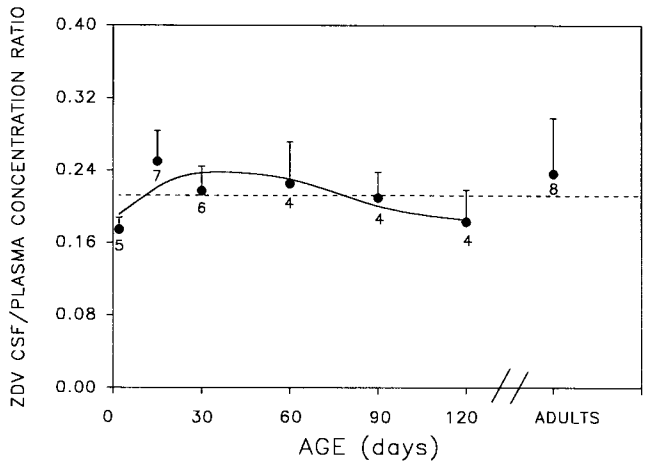

Fig. 1. The change in ZDV CSF/plasma concentration ratio with age in infant macaques is significantly ($P < 0.05$) better described by the nonlinear cubic polynomial model (——) compared with the linear model (---). The numerical values near each data point represent the number of animals studied for that data point, and the error bars represent 1 SD.

and the plasma were determined by high-performance liquid chromatography (11).

When blood and CSF were not sampled simultaneously, the predicted plasma concentration at the time of CSF sampling was obtained by interpolation. For steady-state studies, the data were linearly interpolated; for the iv bolus studies, the data were linearly interpolated after logarithmic transformation of concentration. The ZDV CSF/plasma concentration ratio at 60 min was compared with that at 90 min in both the neonates (paired t test) and the adults (an unpaired t test was used since paired data were unavailable). Since the ZDV CSF/plasma concentration ratios at 60 and 90 min were not significantly ($P > 0.05$) different (Table I), these data were pooled for the adult and neonatal groups, respectively. Then the mean of the ZDV CSF/plasma ratio in the pooled iv bolus adult group was compared with that in the neonates (unpaired t test). In addition, the pooled CSF/plasma ratios from the neonates and adults receiving the iv bolus were compared with the ratios obtained after steady state in the adults (unpaired t test). The effect of age on the ZDV CSF/plasma concentration ratio was examined by regression using both a linear and a nonlinear model.

RESULTS

After iv bolus administration, the ZDV CSF/plasma concentration ratio in both neonates and adults was found not to be significantly affected by time (60 vs 90 min) ($P > 0.05$) (Table I). In addition, no significant ($P > 0.05$) difference was observed in the ZDV CSF/plasma concentration ratio between the adult and the infant groups given the iv bolus (Table II). Moreover, the ZDV CSF/plasma concentration ratios after iv bolus in both neonates and adults were not significantly different from the ratio obtained in adults at steady state after constant-rate infusion (Table I). Because in some of the analyses the sample number is as small as four and the variability large, a type II error cannot be discounted when arriving at these conclusions. However, even if these differences were found to be statistically significant, they are too small to be of clinical importance. As shown in Fig. 1, the CSF/plasma concentration ratio increases after birth ($P < 0.05$) up to 2 weeks of age, then decreases thereafter, to reach a value at 4 months of age which is not significantly ($P > 0.05$) different from that obtained in adults.

DISCUSSION

As has been found in human adults and children (6,7), the ZDV CSF/plasma concentration ratio in the neonate and adult macaques is significantly smaller than unity (Tables I and II). Since the fraction of ZDV bound to plasma proteins in both humans (12) and macaques (11) is small (approximately 0.2), the CSF/plasma concentration ratio should theoretically be close to unity, provided that pseudoequilibrium exists between ZDV influx and ZDV efflux. Deviation of this ratio from unity may be explained by one or both of the following mechanisms. In the first, an active transport process removes ZDV from within the CSF compartment. In the second, ZDV is extensively and irreversibly lost from the CSF compartment by a process such as metabolism. The first mechanism is more likely, since probenecid, a potent inhibitor of anion transport in the kidney tubules (13), increases the CSF/plasma concentration ratio of ZDV in rabbits (14). In addition, no evidence exists that ZDV is extensively metabolized within the CSF compartment. In our experiments, ZDVG was not detected in the CSF samples. Since the pooled ZDV CSF/plasma concentration ratios in both the adult and the neonatal groups receiving the iv bolus were similar to the ratio at steady state in adult macaques, the CSF compartment appears to quickly reach pseudoequilibrium with the plasma compartment. Interestingly, despite differences in the site of sampling (cisternal vs lumbar), the mean ZDV CSF/plasma concentration ratio in the neonates is not significantly different from that obtained in the adults.

Table II. ZDV CSF/Plasma Ratio as a Function of Age and Method of Administration

Age	Method of administration	Sampling time	n	Mean ± SD	P value
Adult	iv bolus	60 and 90 min	8	0.236 ± 0.058*	
Adult	iv infusion	>12 hr	4	0.224 ± 0.094	0.347
Infant	iv bolus	60 and 90 min	30[a]	0.213 ± 0.039*	
Adult	iv infusion	>12 hr	4	0.224 ± 0.094	0.542

[a] All available samples are included in this analysis.
* Not significantly ($P > 0.05$) different.

This suggests that ZDV was homogeneously distributed in the CSF compartment when the samples were obtained.

Although the blood–brain and blood–CSF barriers in mammals are mature at birth, the permeability characteristics of these barriers in neonates often differ from those observed in adults (8). Thus, the small but significant change in the CSF/plasma concentration ratio with age observed in the neonates may result from age-related changes in the permeability of the blood–brain and blood–CSF barriers. Such a pattern of change has been demonstrated previously for methadone and bilirubin in studies conducted in rats (15) and rabbits (16), respectively.

In conclusion, although our data indicate significant changes with age in the CSF/plasma concentration ratio of ZDV after iv bolus administration, these changes are too small to be of clinical significance. Based upon these data, we predict that equivalent ZDV plasma concentrations in adults and neonates will yield equivalent ZDV CSF concentrations.

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REFERENCES

1. R. M. Levy and D. E. Bredesen. Central nervous system dysfunction in acquired immunodeficiency syndrome. In M. L. Rosenblum *et al.* (eds.), *AIDS and the Nervous System,* Raven Press, New York, 1988, pp. 29–63.
2. A. L. Belman, G. Diamond, D. Dickson, D. Horoupian, J. Llena, G. Lantos, and A. Rubinstein. Pediatric acquired immunodeficiency syndrome: Neurological syndromes. *Am. J. Dis. Child.* **142**:29–35 (1988).
3. P. A. Pizzo, J. Eddy, J. Falloon, *et al.* Effect of continuous intravenous infusion of zidovudine (AZT) in children with symptomatic HIV infection. *N. Engl. J. Med.* **319**:889–896 (1988).
4. K. M. Butler, R. N. Husson, F. M. Balis, *et al.* Dideoxyinosine in children with symptomatic human immunodeficiency virus infection. *N. Engl. J. Med.* **324**:137–144 (1991).
5. R. Yarchoan, J. M. Pluda, R. V. Thomas, *et al.* Long-term toxicity/activity profile of 2′,3′-dideoxyinosine in AIDS or AIDS-related complex. *Lancet* **336**:526–529 (1990).
6. R. W. Klecker, J. M. Collins, R. Yarchoan, R. Thomas, J. F. Jenkins, S. Broder, and C. E. Myers. Plasma and cerebrospinal fluid pharmacokinetics of 3′-azido-3′-deoxythymidine: A novel pyrimidine analog with potential application for the treatment of patients with AIDS and related diseases. *Clin. Pharmacol. Ther.* **41**:407–412 (1987).
7. F. M. Balis, P. A. Pizzo, R. F. Murphy, *et al.* The pharmacokinetics of zidovudine administered by continuous infusion in children. *Ann. Intern. Med.* **110**:279–285 (1989).
8. C. E. Johanson. Ontogeny and phylogeny of the blood-brain barrier. In E. A. Neuwelt (ed.), *Implications of the Blood-Brain Barrier and Its Manipulation,* Plenum Medical Book, New York, 1989, p. 179.
9. A. Lopez-Anaya, J. D. Unadkat, L. A. Schumann, and A. L. Smith. Pharmacokinetics of zidovudine (azidothymidine). II. Development of metabolic and renal clearance pathways in the neonate. *J. Acquir. Immune Defic. Syndr.* **3**:1052–1058 (1990).
10. A. Lopez-Anaya, J. D. Unadkat, L. A. Schumann, and A. L. Smith. Pharmacokinetics of zidovudine (azidothymidine). III. Effect of pregnancy. *J. Acquir. Immune Defic. Syndr.* **4**:64–68 (1991).
11. A. Lopez-Anaya, J. D. Unadkat, L. A. Schumann, and A. L. Smith. Pharmacokinetics of zidovudine (azidothymidine). I. Transplacental transfer. *J. Acquir. Immune Defic. Syndr.* **3**:959–964 (1990).
12. J. M. Collins and J. D. Unadkat. Clinical pharmacokinetics of zidovudine. *Clin. Pharmacokinet.* **17**:1–9 (1989).
13. M. A. Hedaya, W. F. Elmquist, and R. J. Sawchuk. Probenecid inhibits the metabolic and renal clearances of zidovudine (AZT) in human volunteers. *Pharm. Res.* **7**:411–417 (1990).
14. R. J. Sawchuk and M. A. Hedaya. Modeling the enhanced uptake of zidovudine (AZT) into cerebrospinal fluid. 1. Effect of probenecid. *Pharm. Res.* **7**(4):332–338 (1990).
15. M. A. Peters. Development of a "blood-brain barrier" to methadone in the newborn rat. *J. Pharmacol. Exp. Ther.* **192**:513–520 (1975).
16. A. M. Jakobson. Bilirubin accumulation by the rabbit choroid plexus in vitro. *Biol. Neonate* **60**:221–229 (1991).