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Effects of Prenatal Exposure to Zidovudine and Lamivudine on Brainstem Auditory Evoked Potentials in Infants from HIV-Infected Women

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ABSTRACT

Long-term *in utero* adverse effects of infants exposed perinatally to antiretroviral drugs are still unknown. The purpose of this study was to determine whether there were differences in Brainstem Auditory Evoked Potential (BAEP) waves and interval interwaves in a group of Zidovudine (AZT) alone or AZT plus Lamivudine (3TC) prenatally exposed infants as the result of a mother with Human Immunodeficiency Virus (HIV) infection; compared with a group of infants not exposed to antiretroviral drugs. Results couls provide an index of neurotoxicity in newborns. Pregnant women were recruited at the first trimester of pregnancy, when they were diagnosed with HIV syndrome. Infants were included in the study if they were exposed prenatally to AZT alone or AZT plus 3TC. BAEP recordings were blinded from each investigator and results compared with a cephalic perimeter-matched control group of non-exposed infants. Comparison of wave latencies showed significant delay of wave I and I-III interwave interval in the AZT-3TC-treated group. The present findings suggest that prenatal exposure to the antiretroviral drugs AZT and/or 3TC is related to increased latencies in wave I and I-III interwave interval. This finding may provide an index of toxicity in lower regions of the brainstem in exposed infants.

INTRODUCTION

Mother to fetus transmission of human immunodeficiency virus (HIV) infection has been reduced since antiretroviral therapy introduction during pregnancy and labor. Zidovudine (AZT) alone or in combination with Lamivudine (3TC) are the most common antiretroviral drugs used in pregnant women [1]. However, long-term *in utero* adverse effects in infants exposed perinatally to antiretroviral drugs remains controversial [1,2]. Some evidence demonstrates that prenatal exposure to AZT and 3TC in mice induces small but significant sensorimotor delay and affects social behavior in offspring [3].

Clinical usefulness of brainstem auditory evoked potentials (BAEP) derives from their low intra- and inter-variability among subjects and their early development in newborns compared to other evoked responses. Different investigations have shown alterations in neonatal BAEP after prenatal exposure to lead [4], cocaine [5], carbamazepine

[6], and other toxins. Thus, the purpose of this investigation was to examine whether there were differences in BAEP waves and interval interwaves in a group of newborns who were products of HIV-infected mothers and prenatally exposed to AZT alone or in combination with 3TC as an index of neurotoxicity in the newborn period. A group of newborns not exposed to antiretroviral drugs served as a control group.

Table I. Clinical characteristics of infants exposed to AZT alone (n=12) or with 3TC (n=25)

		Mean	SD
Weight at birth (g)	AZT	2787.91	371.00
	AZT/3TC	3007.00	365.28
Age at birth (weeks)	AZT	37.97	1.33
	AZT/3TC	38.49	1.08
Apgar score 1 min	AZT	7.66	1.15
	AZT/3TC	8.12	0.72
Apgar score 5 min	AZT	9	0
	AZT/3TC	9	0

METHODS

Pregnant women were recruited at the first trimester of pregnancy when they were seen at the National Institute of Perinatology, a tertiary-level care center for women with pregnancies complicated with HIV infection. Diagnoses were confirmed by ELISA analysis and were included in the Prospective Study of Mothers with HIV and Antiretroviral-Exposed Infants of Mexico City. Mothers in this study were included if they were treated with AZT alone (400 mg/day) or in combination with 3TC (300 mg/day) medication and had no other additional risk factors such as younger than 17 or older tham 35 years of age, diabetes, high blood pressure, infections, drugs abuse, or other potential teratogenic medication. Newborns who met the following elegibility criteria were included: *in utero* exposure to AZT alone and with 3TC exposure during pregnancy by mother with HIV infection. Infants with other risk factors for hypoacusis such as birthweight <1,000 g, Apgar score <7 at 5 min, TORCH infection, meningitis, hyperbilirubinemia (with levels for phototherapy and blood exchange), hypoxia, intraventricular hemorrhage (grade III or with periventricular hemorrhagic venous infarction), or assisted ventilation for >10 days; were rejected from the study. Infants with malformations of head and neck, genetic alterations or metabolic disorders were excluded from the study.

We studied 37 infants prenatally exposed to or AZT/3TC combination; 19 were male and 18 female, all but one born at

term. Infants were exposed to AZT an average of 98.48 ± 52.19 (standard deviation, [SD]) days and 92.92 ± 47.09 days in combination with 3TC. Average of gestational age at birth was 38.32 weeks ± 1.18 with only one premature infant of 35 weeks of gestational age who had adequate birthweight and Apgar scores and was without asphyxia or perinatal complications. Mean characteristics of infants at birth were weight 2,935.94 ± 376.59 g; Apgar score at 1 min 7.97 ± 0.89, and at 5 min, 9 ± 0 without evidence of alterations in blood oxygen concentration. We reported results only from study done most closely to time of birth. Age at time of BAEP study was 40.31 ± 6.72 weeks of conceptional age. A control group with infants born at term and adequate birthweight and Apgar score at 5 min >7 without signs of hypoxia was also selected. Control infants were matched by cephalic perimeter because this parameter has been reported as an important covariant in BEAP measurements.[7] In the control group, we studied 37 infants, 21 males and 16 females. Average gestational age at birth was 39.67 ± 0.99 weeks. Weight at birth was 3,070.02 ± 470.58 g. Apgar score at 1 min was 7.79 ± 0.58, and at 5 min, 8.95 ± 0.20. Age at time of BAEP study was 42.56 ± 4.79 weeks; no parameters had statistically significant differences between groups (*t* test). To study tests performances more clearly, BAEP recordings were blinded from each investigator. No other control group such as HIV infected non-treated mother and exposed infants was studied because we did not see newborns with these characteristics and for ethic reasons [8]. This study was approved by the Research Committee of the Institute and informed consent was obtained from parents of babys.

BAEP determinations were performed in the neontal period during natural sleep after bottle-feeding. Otoscopy was performed prior to BEAP study to disclose vernix caseosa or other sound barriers in the external auditory channel. Each subject was tested with BAEP using ATI system (Buenos Aires, Argentina). Three gold-disk electrodes were placed on the scalp, the negative electrode on the ipsilateral mastoid, positive on the vertex, and neutral on the contralateral mastoid. Interelectrode impedances were < or = 2 kilo-ohms. Electrical activity among electrodes was amplified and averaged over a time base of 10 millisec. Stimulus was administrated through a Telephonics TDH-49 earphone (Telephonics Co., Huntington, NY, USA). Stimuli were presented monoaurally at a rate of 11/sec. Initial presentation intensity was 80 dB (decibels) hearing level and decreased by 20-dB steps to determine neurophysiologic threshold of response. Contralateral ear masking with white noise 30 dB below stimulus intensity was administrated simultaneously. BAEP were recorded and analyzed following recommendations of international standards [9]. The test was performed within a soundproofed room; stimuli were delivered monoaurally and consisted of 100-microsec alternating clicks. Band pass filters were set between 100 and 3,000 Hertz, and stimulus average was 2,024 clicks. The process was repeated at least once to ensure reproducibility of response. Latencies of waves I, III, and V were measured by manual cursor placement at left and right ear recordings separately; I-III, III-V, and I-V interpeak intervals were calculated automatically by neurophysiologic program.

We calculated the average of two trials of faster BAEP responses because this procedure removed possible auditory peripheral alterations [4,6]. Comparisons were performed using two-tailed Student *t* test for independent groups, Pearson correlation coefficients provided measurement of association among AZT and 3TC days of treatment and BAEP waves and interwave-intervals. One-way analyses of variance (ANOVA) of latencies of waves and interwave intervals as dependent variables and group of antiretroviral drug as factor was also performed [10]. Level of statistical significance was =<0.05.

RESULTS

No infants developed HIV infection as confirmed by two negative PCR tests for HIV analyses after birth. Twelve infants were exposed to AZT alone and 25 to AZT/3TC. Clinical characteristics of infants from mothers in therapy with AZT alone or with AZT/3TC did not show significant differences between groups (Table I).

Latencies of BAEP of both exposed and non-exposed infants fell within our laboratory range standards [11], but comparison of waves latencies showed significant delay of wave I in combined AZT and AZT/3TC-treated groups (see Table II). Comparisons of latencies of interwave intervals in collapsed AZT and AZT/3TC to control non-exposed group disclosed significant delay in I-III interval (Table II). Comparison of latencies of BAEP waves and interwave intervals between infants exposed to AZT alone or AZT plus 3TC is shown in Table III and was without significant difference between groups. No significant difference by group of antiretroviral drugs

Table II. Latencies of main waves and interval interwaves of BAEP at 80 dB in infants non-exposed (n=37) with collapsed AZT alone and AZT/3TC (n=37). S.D. = standard deviation. *p<0.05

Wave/Interval (msec)	Non-exposed Mean	SD	AZT-3TC Mean	SD
I	1.73	0.13	1.74	0.25*
III	4.41	0.18	4.37	0.23
V	6.64	0.26	6.61	0.29
I-III	2.68	0.14	2.63	0.21*
III-V	2.22	0.23	2.23	0.25
I-V	4.90	0.26	4.87	0.29

Table III. Latencies of main waves and interval interwaves of BAEP at 80 dB in infants exposed to AZT alone (n=12) and AZT/3TC (n=25)

Wave/Interval (msec)	AZT Mean	SD	AZT/3TC Mean	SD
I	1.77	0.34	1.72	0.21
III	4.33	0.28	4.39	0.20
V	6.67	0.34	6.58	0.26
I-III	2.55	0.25	2.66	0.19
III-V	2.33	0.23	2.19	0.24
I-V	4.89	0.29	4.86	0.29

S.D. = standard deviation.

in latencies of waves and interwave intervals was observed using one-way ANOVA. No significant Pearson correlations among days of treatment and latencies of waves and interval interwaves were found. The electrophysiologic threshold average was within normal range in control and patient groups without differences between groups.

DISCUSSION

Our findings suggested that prenatal exposure to antiretroviral drugs: AZT and 3TC was related to subclinically increased latencies in wave I and I-III interwave interval. The main limitation of the present work is the small sample size and short follow-up period. Thus, results must be considered preliminary and do not permit a strong conclusion.

HIV infection may result in encephalopathy [12] and altered BAEP in children [13], but our infants did not show HIV infection based on PCR determinations. Antiretroviral drugs were used for HIV therapy in pregnant mothers and reduced risk for vertical infection in their offspring, but its effects in postnatal neurodevelopment are still unknown. One report has suggested adverse effects of antiretroviral therapy in infants exposed prenatally to AZT, such as anemia [14]. On the other hand, a follow-up study of uninfected 0-4-year-old children born to HIV-infected women exposed perinatally to AZT showed no adverse long-term effects on developmental and cognitive parameters [15]. However, there are anecdotal cases of congenital anomalies such as neural tube defects in other antiretroviral drug-exposed infants from HIV-infected mothers [16,17].

Mice prenatally exposed to AZT and 3TC showed long-lasting reduction of body weight and maturation of placing and grasping reflexes and pole grasping [3], although differences among species did not allow comparisons, data suggested the possibility that AZT and 3TC could have a neuroteratogenic effect in offspring of HIV-infected mothers when they were administered prenatally, such as we demonstrate in this report. Additional studies are needed to answer this question.

Synchronicity of BAEP may reflect the degree to which axons connect brainstem regions [18]. Infants with AZT/3TC exposure displayed less-well-developed BAEP within lower brainstem regions, suggesting subclinical dysfunction in these auditory centers within the brainstem. To our knowledge, this is the first study to recognize brainstem toxicity of antiretroviral treatment in prenatally exposed newborns. One possible explanation of this fact is that AZT/3TC cause mitocondrial damage in cochlear hair cells [19] and in brainstem neurons, such as that observed in adult patients. Our data suggested that antiretroviral therapy has a preferential effect in the lower brainstem neurons and in auditory nerve and thus, may represent the targets of drug damage. The results presented here suggested the possibility of antiretroviral neurotoxicity during a critical period of auditory system development [20-21].

Subtle differences between controls and prenatal antiretroviral-treated infants suggests a subclinical effect on the auditory pathway in newborns. These functional changes would affect lower brainstem function, may result in pathophysiologic alterations in the auditory system, and suggest that antiretroviral neurotoxicity may be detected by the neurophysiologic methods. These findings must be complemented by studies in larger groups of patients, following them for a longer period postnatally, and measuring antiretroviral levels with pharmacokinetic dynamic tests and during neurologic follow-up for longer study periods.

REFERENCES

1. Mendelbrot, L., Landreau-Mascaro, A., Rekacewicz, C., Berrebi, A., Bénfila, J.L., Borgard, M., Lachassine, E., Barret, B., Chaix, M.L., Bongain, A., Ciraru-Vigneron, N., Crenn-Hébert, C., Delfraissy, J.F., Rouzioux, C., Mayaux, M.J., and Blanche, S.: *JAMA.* 285,2083-2093 (2001).
2. Tuomala, R.E., Shapiro, D.E., Mofenson, L.M., Bryson, Y., Culnane, M., Hughes, M.D., O´Sullivan, M.J., Scott, G., Stek, A.M., Wara, D., and Bulterys, M.: *N. Engl. J. Med.* 346,1863-1870 (2002).
3. Venerosi, A., Valanzano, A., Alleva, E., and Calamandrei, G.: *Teratology* 63,26-37 (2001).
4. Rothenberg, S.J., Poblano, A., and Garza, M.S.: *Neurotoxicology* 15,695-699 (1994).
5. Salamy, A., Eldredge, L., Anderson, J., and Bull, D.: *J. Pediatr.* 117,627-629 (1990).
6. Poblano, A., Belmont, A., Sosa, J., Ibarra, J., Rosas, Y., López, V., and Garza, S.: *J. Child. Neurol.* 17,364-368 (2002).
7. Rothenberg, S.J., Poblano, A., and Schnaas, L.: *Neurotoxicol. Teratol.* 22,503-510 (2000).
8. Public Health Service Task Force *Perinatal HIV Guidelines Working Group* Sept. 22 (2003).
9. Nuwer, M.R., Aminoff, M., Goodin, D., Matsuoka, S., Mauguiere, F., Starr, A., and Vibert, J.F.: *Electroencephalogr. Clin. Neurophysiol.* 91,12-17 (1994).
10. Dawson-Saunders, B. and Trapp, R.G.: *Medical biostatistics* (in Spanish). México City, Manual Moderno (1997).
11. Poblano, A., Garza, M.S., and Ibarra, P.J.: *Bol. Med. Hosp. Infant. Mex.* 52,262-270 (1995).

12. Bruck, I., Tannous-Tahan, T., Rodrigues-da Cruz, C., Flenik-Martins, L.T., Antoniuk, S.A., Rodrigues, M., de Souza, S.M., and de Bruyn, L.R.: *Arq. Neuropsiquiatr.* 59,691-695 (2001).
13. Frank, Y., Vishnubhakat, S.M., and Pahwa, S.: *Pediatr. Neurol.* 8,262-266 (1992).
14. European Collaborative Study: *J. A.I.D.S*. 32,380-387 (2003).
15. Culnane, M., Fowler, M., Lee, S.S., McSherry, G., Brady, M., O´Donnel, K., Mofenson, L., Gotmaker, S.L., Shapiro, D.E., Scott, G., Jimenez, E., Moore, E.C., Diaz, C., Flynn, P.M., Cunningham, B., and Oleseke, J.: *JAMA* 28,151-157 (1999).
16. Richardson, M.P., Osrin, D., and Donaghy, S.: *Eur. J. Obstet. Gynecol. Reprod. Biol.* 93,215-217 (2000).
17. de Santis, M., Carducci, B., and de Santis, L.: *Arch. Intern. Med.* 62:355 (2002).
18. Moore, J.K., Perazzo, L.M., and Braun, A.: *Hearing Res.* 87,21-31 (1995).
19. Marra, C.M., Wechkin, H.A., Longstreth, W.T., Rees, T.S., Syapin, C.L., and Gates, G.A.: *Arch. Neurol.* 54,407-410 (1997).
20. Sininger, Y.S., Doyle, K.J., and Moore, J.K.: *Pediatr. Clin. North Am.* 46,1-14 (1999).
21. Huttenlocher, P.R.: Dev. Neuropsychol. 16,347-9 (1999).