Effect of perinatal short-course zidovudine on the clinical and virological manifestations of HIV-1 subtype E infection in infants

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Abstract

Background: The perinatal short-course zidovudine (ZDV) chemoprophylaxis that can reduce HIV-1 vertical transmission by 51% has been widely practiced in developing countries such as Thailand because of its simpler and less cost. Objectives: To investigate the effects of short-course regimen of oral ZDV for prophylaxis of HIV-1 subtype E vertical transmission among 'break-through' HIV-1 infected infants. Study design: The study analyzed clinical and virological outcomes of 80 infants, whose mothers received ZDV prophylaxis starting at 36 weeks gestation (group Z) and 37 infants whose mothers never received anti-retroviral drugs (group C), at the ages of 1–2, 4–6, and 12 months. Results: Of the 12 HIV-1 infected infants, 5/7 (71.4%) from group Z and 1/5 (20%) from group C progressed to a symptomatic clinical stage by the age 4–6 months. The intersample nucleotide distance of HIV-1 pol reverse transcriptase (RT) sequences of isolates collected at age of 1–2 months from group Z was significantly higher than that from group C (3.34 and 2.92%, P = 0.02). All twelve virus isolates from infected infants were non syncytium inducing (NSI) and macrophage tropic strains; and 5/6 (83.3%) viruses from symptomatic infants were also T-tropic viruses. The symptomatic infants also had significantly higher HIV-1 nucleic acid quantitation than asymptomatic infants. Conclusion: Our results preliminary suggested that infected infants who were perinatally exposed to ZDV may have a more rapid early disease progression with unfavorable viral manifestations than those without exposure to antiretroviral drug. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: HIV-1; Perinatal transmission; ZDV Short course prophylaxis; Subtype E

1. Introduction

The rate of HIV-1 vertical transmission in Thailand with no antiretroviral chemoprophylaxis...
is about 18–24% (Shaffer et al., 1999). The median provincial HIV prevalence reported in sentinel serological surveys of Thai pregnant women increased from 1.0% in 1991 to 2.3% in 1996 (Siriwasin et al., 1998), and Monthly Epidemiological Surveillance Report (2000) in Thailand showed that it has remained around 1.5–1.8% in the past few years. Based on national sentinel surveillance data, it is estimated that there are 18,000 HIV-infected women giving birth each year in Thailand, resulting in a large burden of pediatric AIDS.

In 1994, the Pediatric AIDS Clinical Trials Group (PACTG) Protocol 076 demonstrated that a regimen of Zidovudine (ZDV) given to mothers starting at 14–34 week's gestation, intravenously during delivery and to the newborn for the first 6 weeks of life reduced the risk of perinatal HIV transmission from 25.5 to 8.3% (Connor et al., 1994). However, because of its complexity and cost, this regimen has not been adopted in most developing countries. In 1996, the Ministry of Public Health of Thailand and Mahidol University, in collaboration with The Center for Disease Control and Prevention (Atlanta), initiated a study using a short-course regimen of oral ZDV beginning at 36 weeks of gestation, continuing until delivery with out a neonatal component. This regimen reduced HIV-1 vertical transmission by 51% (from 18.6 to 9.2%) among infants who were not breastfed (Shaffer et al., 1999). The short-course ZDV regimen is more feasible in Thailand because it is less expensive and simpler than the ACTG 076 protocol. From May 1998, all HIV-infected pregnant women who attended at Siriraj Hospital, Bangkok, Thailand, were offered the short-course ZDV regimen.

Transmission of HIV-1 from mother to infant still occurs despite the use of ZDV prophylaxis. One of the main problems in antiretroviral therapy is the selective pressure to escape mutant viruses. Studies have shown that genotypic sequences from infant viral isolates appear to be less diverse than their mother’s, and reflect a minor subset of maternal variants (Wolinsky et al., 1992; Ahmad et al., 1995). Since minor variant viruses were transmitted from mothers to their infants, it is important to know the effect of short-course ZDV on transmitted viruses. We also aimed to investigate the effects of perinatal ZDV on the manifestations and disease progression in 'breakthrough' infected infants.

2. Materials and methods

2.1. Subjects

The subjects were 37 infants [group C (C)] who were born to antiretroviral drug naïve HIV-1 infected mothers (MC) and 80 infants [group Z (Z)] who were born to HIV-1 infected mothers (MZ) that received a short-course ZDV regimen, and attended the special clinic at Siriraj Hospital, Bangkok, Thailand. The two groups of patients were randomly enrolled in the same period from December 1997 to 1999 with written consent from their parents. None of the infants received ZDV therapy during the neonatal period and at the time of blood sampling. They all had normal vaginal delivery at term and were not breastfed. One to two milliliters of blood samples were collected by venepuncture in Vacutainer tubes containing EDTA from all infants at 1–2, 4–6 months, and 1 year of age.

EDTA blood was centrifuged at 1500 rpm for 10 min to separate blood cells from plasma. The plasma was removed and stored at −70 °C. Peripheral blood mononuclear cells (PBMCs) were purified by Isoprep (Robbins Scientific, CA, USA) layer centrifugation. These PBMCs were prepared for proviral DNA detection by polymerase chain reaction (PCR) and virus isolation by coculture method. For PCR analysis, the 10⁶ PBMCs were lysed in 100 μl of PCR lysis buffer containing 10 mg/ml proteinase K (Amresco, OH, USA). Cell suspension was incubated at 56 °C for 1 h and then 95 °C for 10 min to inactivate proteinase K. The cell lysate was then stored at −20 °C until use.

Infants were considered to be HIV-infected, if they had positive results from at least two HIV virological tests, (i.e. DNA-PCR or RNA or by culture performed on separate blood samples, or one positive PCR test plus an AIDS-defining condition according to the CDC classification (Mor-
bidity and Mortality Weekly Report, 1998). Infants were defined as uninfected if two or more results from virological tests were negative, two of which were performed at an age ≥ 1 month, and one of those being performed at an age ≥ 4 months. The clinical staging was classified according to the CDC classification.

2.2. Qualitative and quantitative analysis of the HIV-1 RNA genome in plasma samples

The HIV-1 RNA genome was detected in plasma samples by Nucleic Acid Sequence-Based Amplification (NASBA) using a NucliSens HIV-1 Qualitative/Quantitative kit (Organon Teknika, Boxtel, The Netherlands). The NASBA system comprises a versatile nucleic acid isolation procedure, isothermal amplification and automated homogeneous detection. HIV-1 RNA was extracted from 200 μl of plasma by Boom’s method. The detection limit of the assay was ten RNA copies per input volume. For this study, 200 μl plasma was used, thus yielding a final detection limit of 50 RNA copies per ml. NucliSens kit had been evaluated its sensitivity in HIV-1 RNA quantification of subtype E (Chew et al., 1999; Murphy et al., 2000).

2.3. Polymerase chain reaction amplification

The HIV-1 gag gene was amplified by nested PCR from a DNA lysate of 250 000 PBMCs in all 117 infants. The nested PCR was performed in a two-step reaction, with outer primer pairs (SK380: GAG AAC CAA GGG GAA GTG ACA TAG CAG G and SK 390: TAG AAC CGG TAT ACA TAG TCT CTA AAG GG) and inner primer pairs (SK38: ATA ACC ACC TAT CCC AGT AGG AGA AAT and SK 39: TTT GGT CCT TGT CTT ATG TCC AGA ATG G). The amplifications were carried out in an Automated Gene Amp PCR System 9700 (Perkin–Elmer Cetus, CT, USA). The amplified product was 115 bp. The sensitivity was one copy of proviral DNA in 250 000 PBMCs.

The HIV-1 pol gene was amplified for further direct nucleotide sequencing analysis with RNA extracted from plasma by Boom’s method in 12 HIV-1-infected infant-mother pairs 1–2 months after delivery. The entire reverse transcriptase (RT) coding region was amplified using a RT-PCR with specific oligonucleotide primers (RT01: GGA CCT ACA CCT GTC AAC ATA ATT G, RT02: GTT GGC TAC TAT TTC CTG TAC T, RTI1: GTT GAC TCA GAT TGG TGT TAC TCT A, and RTI2: GTT CTT CTT GAG CCT TAT TTA TCC C). The 1744 by PCR products were subjected to direct sequencing with sequencing primers RTA: GAC AGC ACC AAA TGG AGG AA, RTB: GAG CTC ATC TAT TGA GCT GG, RTC: GCA GAA GTA CAG AAA CAA GG, RTE: CAT AGT GGG AGC AGA GAC TT, RTR: CAT CTA GCA CTG TTA CTG AT. RTZ: TGA GGA GTT TAC ACA GTT GC.

2.4. Sequence analysis of the pol gene

The nucleotide sequences of the HIV-1 pol gene (encoding RT region) were translated into the corresponding amino acid sequences and aligned by using DNASIS version 2.1 (Hitachi Software, Japan). Phylogenetic analysis was performed using software MEGALIGN (LASERGENE99) and PAUP4ASB. The nucleotide distance matrices were calculated using Kimura’s two-parameter method. Phylogenetic trees were constructed using the neighbor-joining method. The number of synonymous substitutions per synonymous site (ds) and number of nonsynonymous substitutions per non-synonymous site (dn) were calculated using the Jukes–Cantor one-parameter model as implemented in the MEGA version 1.01.

2.5. Quantitation of HIV-1 proviral DNA by using competitive PCR

To quantitate the amount of HIV-1 proviral DNA in the PBMCs from the 12 HIV-1 infected infants and their mothers, the competitive PCR (cPCR) assay was used (Menzo et al., 1992). The wild-type template was coamplified with various known amounts of competitor DNA (25, 50, 75… copies) using HIV-1 gag-specific primers (SK380–SK390 and SK38–SK39). After nested PCR, both amplification products were run on 10% polyacry-
lamide gel at 150 V for 90 min in order to separate the 115 bp wild-type DNA from the 97 bp competitor DNA product. After gel electrophoresis and ethidium bromide staining, the relative intensities of each lane of DNA were measured and the area of the peaks calculated by GEL-PRO analysis computer software. The wild-type area and the competitor area ratios were calculated for each lane and plotted against the number of copies of competitor DNA. Finally, the amount of wild-type DNA was calculated from a standard curve at a ratio = 1.

In the competitive PCR assay, each amplification was carried out on a total volume of 50 μl using the same conditions as in nested PCR for gag gene amplification. Cell lysate from 1.5 × 10^5 PBMCs was coamplified with 5 μl of a known number of copies of competitor (25, 50, 75, … copies).

2.6. Virus isolation

The viruses were isolated by a coculture technique from freshly isolated PBMCs cocultured with 2 × 10^6 PBMCs from healthy donors that had been previously stimulated for 48 h with phytohemagglutinin (PHA) (1 × 10^6). Growth of the cell in culture was supported by RPMI 1640 media that had been supplemented with 2 mM L-glutamine, 15% heated-inactivated fetal bovine serum, 100 μl/ml penicillin, 100 μg/ml streptomycin and 10 U/ml interleukin 2 (IL-2). The cultures were incubated at 37 °C, in 5% CO_2. The culture was maintained for 4 weeks and supernatant was collected once a week to test for the presence of p24 HIV-1 antigen by Vironostika® HIV-1 Antigen assay (Organon Teknika); individual isolates were then collected, stored at −80 °C, and subsequently used to evaluate tropism and syncytium inducing (SI) activity.

2.7. Viral phenotype analysis

Primary monocyte-derived macrophage (MDM) cells were obtained by plastic adherence from pooled PBMC of healthy donors, resuspended as 5 × 10^6 cells per ml in medium supplemented with 10% human serum. Five ml of this were added to a 25 cm^-1 flask and incubated at 37 °C for 3–5 days until a layer of adherent macrophage appeared. Then, non-adherent cells were removed and washed four times with phosphate buffer saline (PBS) and fresh medium was added to the flask. MT-2 is an HTLV-1 transformed T cell line that was used to test for the SI phenotype. Sup T-1 is a T leukemia cell line and was used to test T cell line infection. Each cell line was resuspended at 2 × 10^5 cells per ml in RPMI medium

2.8. Statistical analysis

All statistical calculations were done with the SPSS/PC+ software (version 5.0; SPSS Inc., Chicago III.). Results of DNA PCR and qualitative RNA NASBA assays from infants born to HIV-1-infected mothers were compared using McNemar’s test. P < 0.05 was consider statistically significant. The nonparametric Mann–Whitney test was used to compare differences between sample means.

2.9. GenBank accession numbers

All sequences reported here have been deposited in GenBank and were given accession no. AF283698-AF283701, AF283703-AF28704, AF368811-AF368819, AF368824-AF368825, AF368827, and AF368834-AF368840.

3. Results

Of the whole cohort, there were 12 HIV-1 infected infants, seven in group Z (Z11, Z13, Z24, Z27, Z44, Z48, and Z51) and five in group C (C3, C10, C13, C21, and C46). The vertical transmission rates of HIV-1 subtype E in group C and Z were 13.5% (5/37) and 8.75% (7/80), respectively. At the age of 1–2 months, HIV-1 proviral DNA was positive only in 6/117 or 5.1% (C10, Z24, Z27, Z44, Z48, and Z51) but all infected infants (12/117 or 10.2%) were positive for HIV-1 RNA detection. All six infants, who were initially negative for DNA-PCR at 1–2 months-old, became positive for DNA-PCR at the age of 4–6 months.
(C3, C13, C21, C46, Z11, Z13) and their average age of the first sample collection was 37 days. These 12 infants were confirmed as having HIV-1 infection by HIV-1 coculture and persistent seropositivity at 18 months of age. The Nuclisense qualitative HIV-1 RNA detection assay was significantly more sensitive than the DNA-PCR assay for the 1–2 month-old samples, \( (P = 0.031) \).

### 3.1. Clinical staging of HIV-1-infected infants

The clinical category of all 12 infants was classified as EN at the age of 1–2 months. There were 6/12 (50%) infants who developed symptoms at the age 6 months (Table 1). Of these rapid disease progression, five (83.3%) were infants in group Z (Z11, Z24, Z44, Z48, and Z51) and one was in group C (C10). This revealed that more infants in group Z (5/7; 74%) than in group C (1/5; 20%) developed symptoms by 4–6 months \( (P = 0.02) \). The clinical symptoms of these infected infants included oral thrush (4/6; 66.67%), dermatitis (1/6; 33.33%), splenomegaly (5/6; 83.33%), hepatomegaly (83.33%), and lymphadenopathy (3/6; 50%).

### 3.2. Quantitation of HIV-1 proviral DNA and RNA genome

HIV-1 proviral DNA in PBMCs of the 12 HIV-1 infected infants and their mothers at 4–6 months after delivery were quantified by competitive nested PCR and the data is shown in Table 1. The mean quantity of HIV-1 proviral DNA in the 12 HIV-1 infected infants and their mothers was 278 copies per \( 1.0 \times 10^6 \) cells (range from 140 to 407 copies per \( 1.0 \times 10^6 \) cells) and 270 copies per \( 1.0 \times 10^6 \) cells (range from 133 to 460 copies per \( 1.0 \times 10^6 \) cells), respectively. The mean HIV-1 proviral DNA in the five HIV-1 mothers in group C (291 copies per \( 1.0 \times 10^6 \) cells) was not significantly higher than those of the seven mothers in group Z (255 copies per \( 1.0 \times 10^6 \) cells). However, the mean quantity of HIV-1 proviral DNA in the

<table>
<thead>
<tr>
<th>Sample code</th>
<th>DNA PCR/QL RNA result at 4–6 m (1–2 m)</th>
<th>Staging 4–6 m(^a)</th>
<th>Proviral DNA Qt at 4–6 m copies per 1.0 ( \times 10^6 ) cells (mother’s)(^b)</th>
<th>Plasma RNA viral load at 4–6 m log copies per ml (mother’s)(^c)</th>
<th>HIV-1 biotype(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3</td>
<td>+(-)/(++)</td>
<td>EN</td>
<td>267 (133)</td>
<td>5.81 (3.63)</td>
<td>M, NSI</td>
</tr>
<tr>
<td>C13</td>
<td>+(-)/(++)</td>
<td>EN</td>
<td>200 (380)</td>
<td>4.38 (5.11)</td>
<td>D, NSI</td>
</tr>
<tr>
<td>C21</td>
<td>+(-)/(++)</td>
<td>EN</td>
<td>187 (207)</td>
<td>4.11 (4.25)</td>
<td>M, NSI</td>
</tr>
<tr>
<td>C46</td>
<td>+(-)/(++)</td>
<td>EN</td>
<td>140 (460)</td>
<td>3.23 (4.40)</td>
<td>M, NSI</td>
</tr>
<tr>
<td>Z13</td>
<td>+(-)/(++)</td>
<td>EN</td>
<td>160 (140)</td>
<td>4.45 (4.12)</td>
<td>M, NSI</td>
</tr>
<tr>
<td>Z27</td>
<td>+(+)/(++)</td>
<td>EN</td>
<td>347 (227)</td>
<td>3.23 (3.4)</td>
<td>D, NSI</td>
</tr>
<tr>
<td>C10</td>
<td>+(+)/(++)</td>
<td>A</td>
<td>407 (273)</td>
<td>5.23 (5.31)</td>
<td>D, NSI</td>
</tr>
<tr>
<td>Z11</td>
<td>+(-)/(++)</td>
<td>A</td>
<td>367(313)</td>
<td>5.57(5.21)</td>
<td>M, NSI</td>
</tr>
<tr>
<td>Z24</td>
<td>+(+)/(++)</td>
<td>B</td>
<td>293 (347)</td>
<td>5.51 (5.24)</td>
<td>D, NSI</td>
</tr>
<tr>
<td>Z44</td>
<td>+(+)/(++)</td>
<td>A</td>
<td>380 (253)</td>
<td>6.32 (4.21)</td>
<td>D, NSI</td>
</tr>
<tr>
<td>Z48</td>
<td>+(+)/(++)</td>
<td>A</td>
<td>273 (260)</td>
<td>5.31 (4.92)</td>
<td>D, NSI</td>
</tr>
<tr>
<td>Z51</td>
<td>+(+)/(++)</td>
<td>A</td>
<td>313 (247)</td>
<td>6.23 (5.45)</td>
<td>D, NSI</td>
</tr>
</tbody>
</table>

\(^a\) Clinical stage of 12 infected infants at 1–2 m (all are at EN stage).

\(^b\) Mean proviral DNA quantitation of seven Z infants five C infants, seven MZ mothers, five MC mothers were 305, 240, 255, and 291 copies per 1.0 \( \times 10^6 \) cells, respectively.

\(^c\) Mean HIV-1 plasma RNA of seven Z infants, five C infants, seven MZ mothers, and five MC mothers were 5.21, 4.55, 4.65, 4.54 log copies per ml, respectively.

\(^d\) HIV-1 biotype of each HIV-1 isolate was tested by these cell types: MT2 cell line for SI/NSI ability, SupT1 cell line for T-cell tropism, primary macrophage for M-tropism.
seven infants in group Z (305 copies per 1.0 × 10⁶ cells) was significantly higher than those of the five infants in group C (240 copies per 1.0 × 10⁶ cells). Moreover, the six symptomatic infants had a significantly higher quantity of proviral DNA than the six asymptomatic infants [338 and 216 copies per 1.0 × 10⁶ cells, respectively, (P = 0.009).

The mean amounts of plasma HIV-1 RNA genome in group C versus group Z infants and group MC versus group MZ mothers were 4.55 versus 5.21 and 4.54 versus 4.65 log copies per ml, respectively. There was no significant difference in viral load between the two groups of both infants and mothers. But the HIV-1 viral load of the six symptomatic infants was significantly higher than that of the six asymptomatic infants (5.69 and 4.2 log copies per ml, P = 0.006). Also, these infected infants who showed earlier symptoms had a greater quantity of positive proviral DNA detected at the age of 1–2 m (Table 1).

3.3. HIV-1 phenotype of infected infants

All of the 12 isolates from infected infants were non-SI viruses (NSI) identified by their inability to infect and induce syncytium in the MT2 cell line, but all were able to infect and replicate in primary macrophage cultures. In addition, 5/12 (41.67%) of the isolates from symptomatic infants were also able to infect the T cell line.

3.4. Nucleotide sequence analysis of pol gene (RT)

The multiple alignments of the 1680 nucleotide sequences of the entire RT coding region of the HIV-1 pol gene from 12 HIV-1-infected mother-infant pairs (MC3&C3, MC10&C10, MC13&C13, MC21&C21, MC46&C46, MZ11&Z11, MZ13&Z13, MZ24&Z24, MZ27&Z27, MZ44&Z44, MZ48&Z48, and MZ51&Z51) at 1–2 months after delivery were clustered in to subtype E and are shown in Fig. 1. All of the isolates in this study were subtype E, as identified by nucleotide sequencing of the pol (RT) region.

In the entire RT sequences from 12 mother–infant pairs, there were no substitutions commonly associated with nucleoside analog resistance (Hirsch et al., 1998). At position 483, there was a replacement of histidine (H) to isoleucine (I), leucine (L), or tyrosine (Y) in six out of 14 isolates from mothers and infants in group Z whereas all of ten HIV-1 isolates from those in group C had only histidine (H) residues at codon 483 of RT gene.

The mean intersample nucleotide sequence distance among mothers in group C was 2.43% (range 1.87–2.92%), significantly less than that in group Z 3.07% (range 2.43–3.55%, Mann–Whitney U-test, P < 0.001). Among all infants, the mean of the intersample nucleotide sequence distance was 3.16% (range 2.25–4.37%). Similar to the maternal data, the mean distance among group C infants was significantly less than that of group Z infants [2.92 (range 2.49–3.49%) vs. 3.34% (range 2.44–437%), P = 0.042]. The mean nucleotide distance of the RT region between each mother–infant pairs was 1.06% (range 0.54–1.57%). The mean nucleotide distance of mother–infant pairs in group C was not different from that of group Z (1.15 vs. 0.99%, P = 0.327).

All of the pol sequences from the 12 mother–infants pairs showed ds/dn ratios of more than 1.0. The mean ds in group C mothers was significantly lower than that of group Z mothers (0.0660 ± 0.014 vs. 0.0874 ± 0.016, P = 0.001). Similarly, the mean of dn values of the mothers in two groups were significantly different (0.0129 ± 0.003 for group C vs. 0.0153 ± 0.0034 for group Z, P = 0.007). The mean ratio of ds/dn was 5.14 for group C mothers and 5.69 for group Z mothers. The mean ds values of infants in group C and group Z were 0.0924 ± 0.0166 and 0.0980 ± 0.016, respectively; P = 0.410, whereas the mean dn value of group C was significantly lower than that of group Z (0.0122 ± 0.003 vs. 0.0160 ± 0.003, P = 0.004). The mean ratio of ds/dn was 7.59 for group C and 6.13 for group Z infants. The ds and dn values were higher in mother and infants in group Z than in group C. The substitutions in the pol region of subtype E viruses in this study were mainly synonymous, resulting in a very high average ds/dn ratio (i.e. ds/dn = 7.59).
Fig. 1. Phylogenetic tree constructed with sequences of 24 subtype E isolates from 12 infected mother-infant pairs (MC3-C3, MC10-C10, MCD-C13, MC21-C21, MC46-C46, MZ11-Z11, MZ13-Z13, MZ24-Z24, MZ27-Z27, MZ48-Z48, MZ51-Z51). The tree was constructed by Clustal in Megalign, DNASTAR and Neighbor Joining, Bootstrap in PAUP4A5B software programs. The prototype sequence of A, B, C, D, E, F, H, and J are U455, HXB2, Cin2106, Dug11, CM240, Fbr02, Hcf05, J928. The scale bar represents the percentage of nucleotide sequence divergence. The tree is unrooted and branch length is proportional to the number of nucleotide change.

4. Discussion

The perinatal short-course ZDV regimen, which is widely used in Thailand to reduce perinatally transmitted infection, may have an effect on the progression of disease in perinatally infected infants. Mother-to-infant HIV-1, (mostly subtype E), transmission is a major health problem in Thailand.

Perinatally HIV-infected infants experience a shorter incubation time and more rapid disease progression than do HIV-infected adults (Galli et al., 1995). Most HIV-infected infants will have clinical symptoms of infection during the first year of life and up to 16% die before their fourth birthday because of rapid destruction of the immune system and development of opportunistic infections (Wilbert et al., 1998). A few studies have reported more rapid disease progression among perinatally infected infants born to HIV-infected mothers treated with ZDV. There were also some reports from Thailand that about 20–40% of perinatally HIV infected Thai children, whose mothers were drug naïve, developed AIDS within the first year of life (Thisyakorn et al., 1995; Sirisanthana et al., 1999). The majority of these Thai children with symptomatic HIV infection presented with pneumonia, diarrhea, oral candidiasis, lymphadenopathy, or hepatosplenomegaly, and dermatitis. In our study, we also found similar symptoms including lymphadenopathy, hepatosplenomegaly, oral
thrus, and dermatitis in 50% (6/12) of infected infants at age of 6 months, which most of them (5/6; 83.33%) were in group Z. At age of 12 months, two of these children developed pneumonia and no HIV-1 related symptoms had been presented in the other 50% as asymptomatic infected infants (data not shown). Hence, the percentage of HIV-1 infected infants in group C that developed symptoms in the first year of life would be 20% (1/6), which was not different from those reported before (Thisyakorn et al., 1995; Sirisanthana et al., 1999). However, more symptomatic findings (5/6; 83.33%) were presented in infected infants in group Z.

Intrauterine infected infants had a two-fold risk of progression to AIDS or death by 12 months of age compared with those infected intrapartum (De Souza et al., 2000; Martino, 1999). A Short-course ZDV regimen starting at 36 weeks’ gestation mainly reduces intrapartum transmission but not intrauterine transmission of HIV-1 (Shaffer et al., 1999). From our cohort it is possible that most of the ‘breakthrough’ infected infants in group Z had been infected in utero before the mothers had started short-course regimen ZDV and, therefore, progressed faster, as supported by earlier studies (De Souza et al., 2000; Martino, 1999). Intrauterine infection should produce positive virological testing in the first 48 h of life. Unfortunately, we don’t have this data to confirm that the infants with faster progression had in utero infection. However, we found that of six early symptomatic infected infants, 4/5 infected infants in group Z and 1/1 infected infants in group C were tested HIV-1 proviral DNA PCR positive at age 1–2 months. This evidence preliminary suggested that transmission of HIV-1 intrauterine route might be a selected pathway in infected infants of group Z due to ZDV effect. The amount of proviral DNA in the PBMC and the plasma viral load at 4–6 months of age in infants in the group with earlier symptoms was significantly higher than those in the asymptomatic group.

Moreover, the biotype of the viruses isolated from 5/6 (83.3%) of the symptomatic infants were dual tropic and NSI strains. The relationship between the SI phenotype of HIV-1 isolates and clinical progression of disease in adults has been well documented (Martino, 1999; Tersmette et al., 1988, 1989a,b; Cheng-Mayer et al., 1988). In contrast to report observed in adults, the NSI phenotype in perinatally infected infants did not correlate with asymptomatic, slowly progressing HIV infection. Infected infants with NSI strains of HIV-1 progressed to symptomatic disease and AIDS within the first year of life (Spencer et al., 1994). De Rossi et al. (1997) reported that most of the viral isolates and viral variants recovered from infected infants within the first 2 months of life are highly tropic for primary monocyte-derived macrophages (MDM). This further supports the notion that monocyte-macrophage tropic variants are selectively transmitted from mother to infant and/or selectively replicated upon transmission. This study supports these previous studies in that all of isolates from the 12 HIV-infected infants obtained during the first 6 months of life were NSI phenotypes and macrophage tropic viruses. Subtype E has been reported with phenotype property switched from NSI to SI phenotype early after seroconversion (< 4 years) in HIV-1 infected adults (Peeters et al., 1999). So, more data of biotype study of HIV-1 isolates at later stage of infection from these children would be needed.

Overall, the average intersample genetic diversity of the RT nucleotide sequences of the 24 HIV-1 subtype E reported in this study was 2.9%. Genetic distances ranged from 3 to 6% for pol sequences, reported from several previous studies (Huang et al., 1998; Quinones-Mateu et al., 1995). Mothers who received short-course ZDV had more genetic diversity of the pol gene than those who did not receive ZDV (3.07 and 2.43%, respectively; P < 0.001). More genetic divergence in the pol gene of HIV-1 isolates from ZDV treated infected mothers showed the effect of selective pressure of the antiretroviral drug (ds/dn ratio in ZDV treated mothers was 5.69). We found that the mean nucleotide distance of the RT region of HIV-1 pol gene among the seven infants exposed to perinatal ZDV was significantly higher than that among unexposed infants (3.34 and 2.92%, P = 0.02). In this study, none of mother–infant pairs who received short-course ZDV had known genotypic resistance to ZDV. Several amino acids
substitutions are found scattered along the entire RT region. Comelissen M. reported that some substitutions can be classified as subtype specific amino acids, e.g. 43E, 173I, and 238R for subtype A. In this study, we found that one isolate (MZ27) contained 43K, two isolates from C21 and Z48 contained 173R and 173M, respectively, (Cornelissen et al., 1997). Six of 24 isolates from C10, MC10, Z11, MZ11, Z27 and MZ27 at position 238 contained Lysine (K), whereas 18 of 24 isolates at position 238 contained Arginine (R).

We preliminary reported here that subtype E infected infants exposed to perinatal short-course ZDV progressed to a symptomatic stage in the first 6 months of life more often than those unexposed. The symptomatic infected infants had larger quantitation of proviral DNA and RNA detected, more of them were positive for proviral DNA at the age of 1–2 months, had increased intersample genetic divergence of the pol (RT) region, and had dual tropic and NSI phenotypic viruses compared with those infected infants who had a slower clinical course and who were mostly born to antiretroviral drug naive mothers. These laboratory results could be predictive markers of disease progression in perinatally infected infants. Due to the limitation of small numbers, limited data time-point and follow-up, there should be further investigation of ‘breakthrough’ infected infants born from perinatal short-course ZDV chemoprophylaxis mothers.

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References


