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Rapid Development of Genotypic Resistance to Lamivudine When Combined With Zidovudine in Pregnancy

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The prevention of mother to child transmission of HIV-1 by zidovudine monotherapy is well known, but increasingly combination antiretroviral therapy is prescribed during pregnancy. In this prospective study, 19 pregnant women with human immunodeficiency virus-1 (HIV-1) infection who elected to take antiretroviral therapy during the second and third trimesters were treated with zidovudine or zidovudine plus lamivudine. Fourteen women treated with zidovudine monotherapy had a mean 0.3 $\log_{10}$ reduction in viral load and a mean 52 × 10^6/L (17%) increase in CD4+ lymphocytes at delivery compared with pre-treatment samples. Genotypic mutations associated with decreased susceptibility to zidovudine were detected in 2 of 10 women at delivery. Five women with more advanced HIV-1 infection were treated with zidovudine plus lamivudine and a mean 1.5 $\log_{10}$ reduction in viral load together with a mean 30 × 10^6/L (33%) increase in CD4+ lymphocytes was observed in this group. However, four of five women in the dual therapy arm had the M184V mutation in the reverse transcriptase gene associated with decreased susceptibility to lamivudine at delivery. We conclude that zidovudine plus lamivudine reduced HIV-1 plasma viraemia to low levels in pregnant women with advanced HIV-1 disease but the rapid development of genotypic resistance to lamivudine indicates that additional therapy is required both for the long-term benefit of the mothers and to prevent the development of resistant virus that may be transmitted to the infant. ***J. Med. Virol. 59:364–368, 1999.***

KEY WORDS: HIV, mother-to-child transmission; zidovudine; lamivudine

INTRODUCTION

It has been known for some time that zidovudine monotherapy improved the short-term survival of patients with ARC or AIDS [Fischl et al., 1987], although long-term studies revealed that there was no survival benefit from the early use of zidovudine in less advanced disease [Concorde Coordinating Committee, 1994; Volberding et al., 1995]. The results of the ACTG 076 study, which showed a 68% reduction in mother to child transmission in women receiving zidovudine monotherapy during pregnancy, were therefore unexpected [Connor et al., 1994]. In countries with sufficient resources, zidovudine administration during pregnancy has rapidly become the standard of care and other epidemiological studies have since confirmed the impact of this landmark study on perinatal transmission [Cooper et al., 1996; Blanche et al., 1997]. Recent studies have shown a substantial reduction in mortality over a 3-year period when zidovudine was combined with didanosine in drug-naive patients, but significantly less benefit was observed when this combination was used in zidovudine-experienced patients [Delta Coordinating Committee, 1996]. This finding lead many centres to reappraise their recommendations for prescribing anti-retroviral therapy in pregnancy. Because the only published pharmacokinetic and safety data for anti-retroviral drugs in pregnancy were for zidovudine [O'Sullivan et al., 1993] and lamivudine [Johnson et al., 1996], this combination was chosen by our clinic for the treatment of human immunodeficiency virus (HIV) in pregnant women with more advanced HIV disease who at that time were defined as women with AIDS or

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TABLE I. Comparison of the Pretreatment and "at Delivery" Immune and Virological Characteristics of Mothers Treated With Zidovudine Monotherapy or Zidovudine Plus Lamivudine

	Zidovudine	Zidovudine + lamivudine	*P*
Number of subjects	14	5	
Mean age (years)	27.5	32	
Genotypes	3A, 2B, 3C, 2G	3A	
Pretreatment			
CD4+ lymphocyte count × 10^6/L (mean)	301 (21.8%)	96 (9.8%)	
(median)	345 (21.5%)	88 (10.9%)	.001
Plasma viral load $\log_{10}$ copies/ml (median)	4.3	4.75	.04
LiPA	WT	WT	
Number of days on treatment (mean)	63.8	118.8	.02
Delivery			
CD4+ lymphocyte count × 10^6/L (mean)	353 (25.7%)	126 (12%)	
(median)	375 (25.5%)	128 (12%)	
Change CD4+ lymphocyte count	+52 (17%) $P = .06$	+30 (31%) $P = .08$	>.05
Plasma viral load $\log_{10}$ copies/ml (median)	3.99	3.33	.2
Viral load reduction $\log_{10}$ copies/ml (mean)	0.3	1.46	.004
LiPA	8 WT	1WT	
	1 K70R/WT	3 M184V	.05
	1 M41L/T215Y	1 M184V/K70R	

a CD4 count of < 350 × 10^6/L or a plasma viral load of > 30,000 copies per ml of plasma.

Since December 1995, we have monitored prospectively the effect of anti-retroviral therapy in a cohort of pregnant women attending this hospital. In addition to measuring CD4 lymphocyte count and plasma viraemia, we have used the Murex HIV-1 line probe assay (LiPA HIV-1 RT) to assess the development of mutations known to confer resistance to zidovudine and lamivudine.

MATERIALS AND METHODS

Subjects

The majority of women in this study were from sub-Saharan Africa and were infected with a non-B subtype of HIV-1. One mother had a 15-month exposure to zidovudine 4 years prior to this pregnancy but the remainder were anti-retroviral naive and the baseline characteristics of the women are summarised in Table I.

Anti-Retroviral Therapy

Zidovudine therapy was 250 mg twice daily from the start of the second trimester. During labour, zidovudine was administered by intravenous infusion with a 1-hr loading dose of 2 mg/kg per hour followed by 1 mg/kg per hour until clamping of the cord. Maternal therapy was then discontinued. Babies were given zidovudine 2 mg/kg every 6 hr for the first 3 weeks of life then switched to co-trimoxazole 900 mg/m^2 three times a week for PCP prophylaxis. Lamivudine 150 mg twice daily was given from the start of the second trimester. An extra oral dose of lamivudine was recommended at the onset of labour. Mothers on dual therapy were advised to continue full anti-retroviral therapy post-partum and thereafter managed as per non-pregnant women.

Resistance Assay

Plasma samples were collected from 19 HIV-1-infected pregnant women at baseline, at delivery and 3 weeks post-partum and were stored at –70°C until use. HIV RNA extraction, cDNA synthesis and polymerase chain reaction (PCR) amplification were undertaken as described previously [Stuyver et al., 1997]. Briefly, 50 μl of plasma were mixed with 150 μl of Trizol (Gibco-BRL, Paisley, UK). After lysis and denaturation, chloroform was added to achieve phase separation and the RNA precipitated with isopropanol. The RNA pellet was resuspended in random prime mixture (pd [N]6) (Pharmacia, Milton Keynes, UK). cDNA synthesis was carried out using 10 units of AMV reverse transcriptase (Promega, Southampton, UK) at 42°C for 90 min. Nested PCR amplification of the target HIV RT gene was achieved using 5 μl cDNA, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 10 μM dNTP, 1 unit AmpliTaq (Applied Biosystems, Warrington, UK). The outer primer sequences were as described in reference 11 and were 5-bio-ATCAGGATGGAGTTCATACCCATCCA and 5-bio-GTACAGTATTAGTAGGACCTACACCTGCTC. The inner primer sequences were 5-bio-CCAAAAGTTAAACAATGGCCATTGACAGA and 5-bio-AGTTCATAACCCATCCAAAG. The PCR conditions were 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min. Forty cycles of amplification were carried out for the outer PCR reaction and 35 cycles for the nested PCR. Amplification of the appropriate sequence was confirmed by the detection of a 650-bp band following electrophoresis of 10 μl of PCR product on a 1% agarose gel. The LiPA was carried out by denaturing 10 μl of nested PCR product by mixing with an equal volume of 0.4 M NaOH/0.1% SDS at room temperature for 5 min. Hybridization of the PCR product was carried out at 39°C for 30 min in 2 ml of hybridization buffer according to the manufacturer's instructions (Murex Biotech, Dartford, UK). The hybridization mixture was then re-

placed with stringent wash buffer and washing carried out at 39°C for 10 min. The buffer was then removed the strips thoroughly rinsed and the streptavidin-alkaline phosphatase conjugate added for 30 min at room temperature. The conjugate was then washed away and the nitroblue tetrazolium and 5-bromo-4-chloro-3-indoyl phosphatase substrates added for 30 min at room temperature. The probes in which hybridization had occurred were visible as a purple-brown precipitate. LiPA HIV-1 RT detected wild-type and mutant genotypes at codons 41, 69, 70, 74, 184, and 215.

Plasma HIV-1 RNA Viral Load Assays

HIV-1 plasma viraemia was monitored in this cohort of patients using commercially available assays. Each baseline maternal sample was evaluated by NASBA [Organon Diagnostics, Cambridge, UK], bDNA [Chiron Diagnostics, Colchester, UK] and HIV-1 monitor [Roche Diagnostics, Welwyn Garden City, UK] to reduce the likelihood of underestimating HIV-1 RNA viral load particularly for non-clade B viruses. The assay giving the highest value at baseline was used for all subsequent comparisons on any given individual.

HIV-1 Subtype Evaluations

Genotyping of HIV-1 sub-type was carried out using the heteroduplex mobility assay as described elsewhere [Delwart et al., 1993].

Statistical Analysis

Median values were compared between groups by the unpaired Mann-Whitney test and within groups by the paired Mann–Whitney test. The number of patients with genotypic resistant mutations in each group were analysed using the Fisher exact test.

RESULTS

The results are summarised in Table I. Fourteen subjects with a mean pre-treatment CD4 lymphocyte count of 301×10^6/L (range $50–410 \times 10^6$/L) and with a median baseline viral load of 4.3 $\log_{10}$ (range 3.05–4.99 $\log_{10}$) received zidovudine monotherapy during pregnancy. The median viral load reduction at time of delivery was 0.3 $\log_{10}$, whilst CD4 counts had increased by a mean of 52×10^6/L (17%) in this group. Five patients in the study received zidovudine and lamivudine dual therapy. The mean baseline CD4 count in these five patients was 96×10^6/L (range $20–150 \times 10^6$/L), the median baseline viral load was 4.75 $\log_{10}$ (range 4.54–5.10 $\log_{10}$) and the median viral load reduction in this group was 1.46 $\log_{10}$ with a 30×10^6/L (31%) increase in CD4 lymphocyte count. The differences between the baseline CD4 counts ($P = .001$), median baseline viral load ($P = .04$), and days on treatment ($P = .02$) between the two groups of patients were all statistically significant, reflecting the more advanced disease in the patients selected for combination anti-retroviral therapy. The virological efficacy of the combination treatment is shown by the 1.5-$\log_{10}$ reduction in baseline viral load ($P < .001$). The virus in the mother with past zidovudine exposure (who received further zidovudine therapy in this pregnancy) remained wild type throughout.

The LiPA HIV-1 RT assay results showed that all baseline samples were wild type. Of the 10 patients who received zidovudine monotherapy during pregnancy, one developed a mixed wild-type/mutant population at position 70 by delivery and another had both M41L and T215Y mutations indicating high level resistance to zidovudine. In four of five patients treated with zidovudine plus lamivudine, the M184V genotypic mutation bestowing resistance to lamivudine was detected before or at delivery. This mutation could be detected as early as 8 weeks into therapy. One patient, in addition to the 184 mutation, had the K70R mutant at delivery that is associated with mild/moderate resistance to zidovudine. In the one patient receiving dual therapy who did not have the M184V mutation prior to delivery, this mutation was detected post-partum. The rate of detection of genotypic mutations between the two groups of patients in this study was significant ($P = .05$).

DISCUSSION

Prior to October 1996, zidovudine monotherapy was recommended to all pregnant women known to be infected with HIV-1 attending our clinic to reduce mother-to-child transmission of the virus. In 1996 the results of the Delta study, a multi-centre, European and Australian study comparing zidovudine monotherapy with zidovudine combined with didanosine or zalcitabine in drug-naive and zidovudine-exposed subjects were published. This double-blind, placebo-controlled study of more than 3,200 subjects showed a substantial reduction in morbidity and mortality compared with zidovudine alone in patients randomised to receive two nucleoside analogues in combination. Because this effect was reduced significantly by prior exposure to zidovudine, two nucleoside combinations became standard initial therapy in non-pregnant women. Due to the absence of published safety and pharmacokinetic data in human pregnancy for didanosine, stavudine, and zalcitabine, the combination of zidovudine, which is of proven benefit in preventing perinatal transmission of HIV-1, and lamivudine, which is well tolerated and for which limited data in human pregnancy were available, was chosen for the treatment of pregnant women with more advanced disease at our centre. Thus from October 1996 women were offered zidovudine monotherapy if the objective was to prevent HIV-1 mother-to-child transmission and the combination of zidovudine with lamivudine if anti-retroviral treatment for the mother would have been recommended according to clinic guidelines.

Of the 19 women in this study, 14 received zidovudine monotherapy. As in ACTG 076, the viral load reduction was only 0.3 $\log_{10}$. The only mother to transmit HIV in this group had a low plasma viral load (5,653 copies/ml at baseline and 5,458 copies/ml 4 weeks postpartum) but delivered at another hospital and did not

receive peri-partum zidovudine, nor did her baby receive zidovudine in the neonatal period (transmission rate 7.6%). Five women took zidovudine plus lamivudine. These women had more advanced disease, with lower CD4 counts and higher baseline viral loads than the zidovudine group. In untreated patients such viral loads have been associated with a high risk of transmission and even with zidovudine monotherapy the transmission rate was 18% if the plasma viral load was > 10,000 copies/ml [Contopoulos-Ioannidis and Ioannidis, 1998]. Whilst in the ACTG 185 study transmission was 9% if the maternal CD4 T-lymphocyte count was less than 200×10^6/L compared with 3% if the CD4 T-lymphocyte count was greater than 200×10^6/L despite zidovudine therapy [Paediatric ACTG 0185, 1997]. However, by the time of delivery, a mean 119 days on treatment, viral load had fallen by 1.5 $\log_{10}$ to 3.3 $\log_{10}$, whilst viral load in the zidovudine group at delivery was 4.0 $\log_{10}$. Although the numbers are too small to be statistically significant, the fact that none of these mothers transmitted HIV supports the use of combination therapy to prevent mother-to-child transmission in women with more advanced disease than in the ACTG 076 study.

Data on genotypic resistance to zidovudine and lamivudine were available for 15 mothers, 10 in the zidovudine group and all 5 treated with the combination. Only one of the mothers had taken either drug previously and all initially had wild-type genotype at each relevant codon. Although genotypic evidence of zidovudine resistance was seen in only two mothers prescribed zidovudine alone, this was more frequent than seen in the ACTG 076 study. Previously, zidovudine resistance in pregnancy has been described in those mothers who commenced therapy prior to conception [Frenkel et al., 1995]. Despite, or perhaps because of, the 1.5-$\log_{10}$ reduction in viral load in the five mothers on combination therapy, genotypic evidence of resistance to lamivudine was seen in four by the time of delivery and was first detected by as early as 8 weeks into therapy. Although genotypic changes that confer reduced sensitivity to lamivudine appear to protect against zidovudine resistance, one mother had evidence of both lamivudine and zidovudine resistance. Thus the rapid development of lamivudine resistance, which has been noted in non-pregnant subjects [Schuurman et al., 1995], occurs during the relatively short duration of treatment in pregnancy. The higher baseline viral load and longer duration of treatment in this group are likely to have contributed to the development of resistance. These data also show that non-clade B viruses also exhibit the common mutations associated with reduced sensitivity to nucleoside analogues and that LiPA HIV-1 RT is able to detect these mutations. This finding is important as most pregnant women in the world are infected with non-clade B viruses. In this cohort, none of the infants was infected with HIV and although the numbers are small, this combination does seem to be protective despite the development of lamivudine resistance. However, should transmission occur it seems reasonable to assume that the infecting virus is resistant to lamivudine. This resistance would have serious implications for the future management of the infant, impacting both on the number of therapeutic options and probably on the long-term efficacy of anti-retroviral treatment.

The rapid development of lamivudine resistance has important implications for the future management of the mothers and we conclude that as in non-pregnant women, zidovudine plus lamivudine should not be used as an initial therapy when other options exist. This conclusion cannot be extrapolated to the short-term use of zidovudine and lamivudine in the last 2 weeks of pregnancy. Recent guidelines suggest that the criteria for prescribing anti-retroviral therapy in pregnancy should be as per non-pregnant women, although the choice of combination will be directed by the suitability of compounds for use in pregnancy [US Public Task Force, 1998].

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