MTCT REGIMEN CHOICE, DRUG RESISTANCE AND THE TREATMENT OF HIV-1-INFECTED CHILDREN

G Gray, *FCP (Paed)*
Perinatal HIV Unit, University of the Witwatersrand
L Morris, *BSc Hons, DPhil*
AIDS Virus Research Unit, National Institute for Communicable Diseases
J McIntyre, *MRCOG*
Perinatal HIV Unit, University of the Witwatersrand

Mother-to-child transmission of HIV-1 (MTCT) remains a major route of infection in South Africa, where 25% of pregnant women are HIV-infected[1] and an estimated 70 000 - 80 000 HIV-infected infants are born annually. The risk of transmission is highest during labour and delivery, and studies have demonstrated that antiretroviral (ARV) treatment, initiated either antenatally or in the intrapartum period, can result in significant reductions in transmission of HIV-1 from mother to infant. Widespread introduction of these regimens to prevent MTCT in South Africa will have a major impact on controlling perinatally acquired HIV infection. The presence of ARV therapy can alter viral ecology, and if the therapy allows ongoing viral replication, drug-resistant variants may become selected as long as the drug is administered. There has been some concern that the use of ARV monotherapy for the prevention of MTCT, including zidovudine (ZDV) or nevirapine (NVP) used alone, could result in the development of drug resistance with potential implications for perinatal transmission, the choice of therapy for the HIV-infected infant and future maternal therapeutic options. In the USA and other developed countries, the detection of ZDV or other resistant variants has not been found to be associated with an increased risk of MTCT[2] and, while perinatal transmission of a resistant variant has been reported, this occurrence appears to be infrequent and unusual.[2-4] In a substudy of the Women and Infants Transmission Study Group (WITS), univariate analysis showed that ZDV resistance was not significantly associated with transmission, but when adjusted for duration of ruptured membranes and total lymphocyte count, resistance mutations conferred an increased risk of transmission.[5] However, these women were more likely to have higher viral loads and lower CD4 counts and needed to be on ARV treatment for their own health.

ANTIRETROVIRAL DRUG RESISTANCE TESTING

Genotypic and phenotypic assays are available for assessing ARV drug resistance. Genotypic assays detect specific point mutations in the HIV genome that are associated with phenotypic resistance. These are most often detected by direct sequencing of the protease (PR) and reverse transcriptase (RT) genes following PCR amplification. Plasma samples are recommended for genotyping and in general a viral load of at least 1 000 copies/ml is required. These assays can detect up to 25% of resistant variants in mixtures of mutant and wild-type genomes. However, it is becoming increasingly important to be able to detect populations that occur at a lower frequency, so-called minority populations, which can rapidly become dominant once drug pressure is applied. Genotypic testing is the most frequently used method as it is rapid and less expensive than phenotyping. However, it is an indirect measure of resistance and provides no indication of the degree of phenotypic resistance to a given drug or combination of drugs (cross-resistance). A compendium of resistance mutations for currently used ARVs is available on-line together with interpretation algorithms (**www.hivdb.stanford.edu/hiv/**), although expert clinical opinion together with a detailed treatment history is often required for accurate interpretations, particularly for patients failing complex regimens.

Phenotypic assays measure the susceptibility of a virus to a specific drug in a culture assay. In general an isolate able to replicate in 5 - 10-fold higher concentrations of drug would be considered to show phenotypic resistance. These assays have been problematic in the past as they relied on the ability to isolate HIV from individual patients. Nowadays assays based on recombinant DNA technology are used, where the gene regions encoding RT and PR are amplified and inserted into a laboratory strain. These hybrid viruses are grown in the presence of increasing concentrations of various drugs and the levels of viral replication monitored. These assays are time-consuming and expensive but provide a more direct measure of resistance as well as useful information on the levels of

resistance and cross-resistance. Phenotypic resistance tests are also used to determine whether previously unknown treatment-associated mutations confer resistance. While these assays are generally easier to interpret than assays for genotypic resistance, the clinical significance of low levels of phenotypic resistance remains uncertain.

PREVALENCE AND SPECTRUM OF ANTIRETROVIRAL RESISTANCE MUTATIONS

NUCLEOSIDE REVERSE-TRANSCRIPTASE INHIBITORS (NRTIs)

The prevalence of ARV drug-resistant variants in therapy-naïve HIV-infected pregnant women varies geographically. The prevalence will depend on background use of ARV therapy within the population and the type of assay used to measure resistance.[6] In the USA and Europe, where the predominant HIV sub-type is B, rates of primary resistant variants in the reverse transcriptase gene (which drugs like ZDV, 3TC, ddI, d4T and nevirapine target) were between 10% and 23%.[7] Resistance occurs in these populations because ARVs have been in use for many years and is not related to subtype. In South Africa, where the predominant subtype is C, and where ARVs are not readily available for widespread use, drug-resistant viruses do not appear to be prevalent in HIV-infected pregnant women.[8] The prevalence of resistant variants in pregnant women will vary depending on the population studied, i.e. whether or not ARVs are in widespread use. In the USA, a study confirming the efficacy of ZDV in preventing MTCT (ACTG076 study), no high-level resistance to ZDV was detected.[2] In contrast, among women who were receiving ZDV for health reasons prior to 1994, any ZDV resistance was detected among 25% of women, and high-level resistance was detected in 10% of the isolates. Yet there have been a few perinatal transmissions in heavily treated women.

Rapid development of resistance to 3TC has been reported among HIV-infected adults who have received dual nucleoside therapy without other agents. In a small study, 4 out of 5 women treated with ZDV/3TC in pregnancy had the M184 V 3TC resistance mutation detected by delivery.[9] Similarly, in a French cohort who had 3TC added to a ZDV regimen at 32 weeks found an increase of the M184V mutation from baseline (2% at baseline to 39% at 6 weeks *post partum*).[10] Resistance has also been assessed in the PETRA (Perinatal Transmission) study where ZDV and 3TC were used. In PETRA arm A, where women received ZDV and 3TC antenatally for 4 weeks, during labour and delivery, and *post partum* for 1 week, 2 women were found to have the M184V; these 2 women did not transmit the virus to their infants.[10] Because of the profound reduction in transmission rate using this regimen, short-term treatment with dual nucleoside therapy may be considered in pregnant women for MTCT prophylaxis. In a study conducted at the Chris Hani Baragwanath Hospital, where women were randomised to receive 4 weeks of ZDV or D4T or DDI or a combination of d4T and ddI, no resistance was seen in HIV-infected infants at 6 weeks of age.[11]

NON-NUCLEOSIDE REVERSE-TRANSCRIPTASE INHIBITORS (NNRTIs)

The only NNRTI that has been tested in MTCT so far is NVP. Studies conducted in Uganda and in South Africa have demonstrated that drug-resistant mutations were present in maternal and infant viral sequences *after* NVP was administered as part of an MTCT regimen.[12,13] In Uganda, where a single dose of NVP was administered to HIV-infected women, these mutations were not associated with failure to prevent MTCT,[8] i.e. they did not compromise the efficacy of NVP in preventing MTCT. This study showed that the rate of development of NVP resistance was approximately 18% in mothers 6 weeks after exposure, and that these resistant mutations were undetectable 18 months after delivery. In the South African Intrapartum Nevirapine Trial, selection of resistance mutations was determined in a trial of two doses of NVP compared with 7 days of zidovudine/lamivudine (ZDV/3TC) for the prevention of MTCT of HIV. HIV genotyping was performed on plasma samples collected 4 - 6 weeks *post partum* from women receiving NVP and ZDV/3TC and HIV-1-infected infants born to mothers who had received NVP. HIV-1 genotyping was then repeated on samples taken 9 -12 months *post partum*. No ZDV or 3TC mutations were detected in the women who received the multiple-dose ZDV/3TC regimen. NVP resistance mutations were detected in 74 (67%) of the 111 women who had received 2 doses of NVP. The predominant NVP mutations found were K103N (62%) and Y181C (45%). Studies of the infected infants (4 - 6 weeks of age) demonstrated NVP mutations in 53%. The predominant mutation was Y181C, which was present in 53% of the infected infants. Paired data from 26 mother-infant pairs suggested that in a few instances NVP-resistant virus may have been transmitted through breast-feeding. However, the presence of these mutations is unlikely to have been the reason for infection in the child, as in the absence of NVP these variants have no selective advantage but merely reflect what was present in the mother at the time of transmission. Long-term follow-up samples showed that in women with NVP resistance mutations detected at 4 - 6 weeks, most virus had reverted to wild type by 13 months. In summary, the use of a 2-dose maternal regimen for preventing MTCT is associated with an increased frequency of resistant variants and the results from the SAINT data favour the use of single-dose NVP.

RESISTANCE TESTING RECOMMENDATIONS

The International AIDS Society-USA Panel and Euroguidelines Group for HIV resistance recommend that all pregnant women with detectable HIV RNA levels have resistance testing performed, even if they are antiretroviral-naïve. This is to maximise the response to ARVs during and after pregnancy and to aid in the choice of antiretroviral therapy should the infant be HIV-infected. However, no data show that routine resistance testing decreases MTCT or improves maternal outcome, and until further data become available, resistance testing for HIV-infected pregnant women should be the same as for non-pregnant adults. The situation may be different in developing countries where single-dose NVP is likely to be widely used. Table I is a guide for resistance testing in HIV-infected pregnant women.

TABLE I. INDICATIONS FOR RESISTANCE TESTING IN HIV-INFECTED PREGNANT WOMEN

- Acute infection/seroconversion
- Virological failure with persistently detectable HIV RNA levels while receiving antenatal therapy, or sub-optimal viral suppression after initiation of highly active antiretroviral therapy
- Those with a likelihood of having resistant virus based on the community prevalence of resistant virus, e.g. known drug resistance in sexual partner
- Previous exposure to single-dose NVP?

HIV RESISTANCE ASSAYS IN PEDIATRICS

In HIV-infected adults, resistance assays may prove useful in guiding initial therapy and in changing failing regimens, but their value in children has not been established and expert clinical interpretation is required. Standardisation of assays used will be necessary before these assays can be incorporated into the clinical care of children. If resistance testing is performed in infants, these tests should be done while the infant is being exposed to the ARV therapy. In the absence of drug pressure, wild-type virus will dominate and may mask the presence of resistant virus.

As viral replication is higher in infants than in adults, and this puts them at increased risk of developing ARV resistance. Indeed, in HIVNET 012 children had a higher frequency of NVP resistance mutations than their mothers. Furthermore, mutational patterns to NVP often differed between infants and their mothers, suggesting that other factors might impact on the development of resistance mutations in children, such as the immature immune response. Further studies on the evolution of ARV in children are warranted.

THE CHOICE OF REGIMENS FOR HIV-INFECTED INFANTS EXPOSED TO MTCT REGIMENS

The use of ZDV and NVP as single agents to prevent MTCT is recommended. The management of HIV infection in infants is rapidly evolving and increasingly complex. Where possible, ARV therapy in an infant under 12 months of age should be initiated in consultation with a specialist in the treatment and management of HIV infection. The choice of ARV regimens for the initiation of treatment should take into account the limitation of future treatment options, as well as the presence or future development of ARV resistance. There are potential problems with early initiation of therapy, including short-term and long-term side-effects, inadequate data on drug dosing, pharmacokinetics and safety. The initial ARV regimen chosen for HIV-infected infants could theoretically be influenced by the ARV regimen the mother was exposed to during pregnancy. If she received ARV treatment during pregnancy, either to reduce MTCT or for her own disease, there is a possibility that she may transmit resistant virus to her baby should he or she also become infected. This is a particular problem when NVP or 3TC has been used as part of a non-suppressive regimen, as resistance to these drugs can be induced rapidly by a single-point mutation.

Data from the USA do not suggest that the ARV regimen for the newly diagnosed HIV-infected child should routinely be chosen on the basis of the maternal regimen. If maternal ARV drug resistance is documented or suspected, resistance testing of infant viral isolates may be considered in order for the clinician to assist in the choice of the initial ARV regimen. It is not known whether ARV choices for infants who have been exposed to non-suppressive ARV regimens used to prevent MTCT should be modified. The efficacy of first-line highly active antiretroviral therapy (HAART) treatment regimens containing NVP or 3TC for infants who are infected despite prophylaxis containing these drugs requires further research. In the meantime, prior administration of short-course ZDV/3TC should not preclude use of these drugs as part of combination ARV drug regimens for treatment of HIV-infected children, particularly those initiating therapy at 12 months of age or older (at which time wild-type virus is likely to predominate). There are no data on the efficacy of NVP used in combination with other ARVs in the treatment of HIV-infected infants after having received NVP as part of preventing MTCT. Until such data become available, it may be advisable to avoid using NVP or efavirenz in the initial ARV regimen. If ZDV monotherapy was used in MTCT, available data support the use of ZDV as part of combination therapy in HIV-infected infants.

THE MANAGEMENT OF HIV INFECTION IN INFANTS AND CHILDREN EXPOSED TO MTCT REGIMENS

Once HIV infection has been confirmed in the infant, any ARV therapy that has been used for prophylaxis for MTCT should be stopped. In general, the local South African guidelines do not recommend treating infants under 3 months of age with ARV therapy unless they fulfil the indication for starting therapy (clinical category B or C, or CD4% < 20%). If the clinician is considering resistance testing, this should be undertaken while the infant is still receiving the prophylaxis to assess the prevalence of resistant mutations and to provide information that will guide the clinician in his/her choice of the initial regimen. If the child or infant is not on any prophylaxis, there is no value in doing resistance testing, and a detailed maternal drug history will aid the clinician in the choice of the initial regimen.

CONCLUSION

Perinatally acquired HIV is a potentially preventable disease and the introduction of interventions to prevent MTCT in South Africa will help control paediatric HIV. Further research is required into the prevalence and significance of ARV drug resistance in MTCT. Although evaluations of MTCT trials have demonstrated the emergence of NVP-specific genotypic mutations in treatment-naïve HIV-infected women after a single dose regimen, the ability to detect these mutations decreases over time, and they are undetectable 18 months *post partum*. There are currently no data to suggest that a single dose of NVP used for the prevention of MTCT will affect the efficacy of a NVP-containing treatment regimen for HIV-infected children.

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