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MINIREVIEWS

Multiple Effects of the M184V Resistance Mutation in the Reverse Transcriptase of Human Immunodeficiency Virus Type 1

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The initial use of nucleoside reverse transcriptase inhibitors (NRTIs) in treatment of human immunodeficiency virus (HIV) disease, followed by highly active antiretroviral therapy, has significantly diminished HIV-related morbidity and mortality (22). However, antiretroviral therapy has also led to the emergence of drug resistance, potentially leading to virological and clinical failure. This problem is offset to some extent by the finding that drug-resistant viruses may have a measurable replication disadvantage in comparison to wild-type strains in the absence of drug pressure (26). This diminution in viral replication capacity or fitness is the result of resistance-conferring mutations in the reverse transcriptase (RT) and protease enzymes of HIV that affect their function; these mutations are amplified and/or selected by antiviral drug pressure.

Therapeutic regimens containing lamivudine (3TC) have been shown to be highly effective in the treatment of HIV-infected patients, despite the fact that a single mutation at position 184 involving a transition from methionine to valine (M184V) confers a loss of susceptibility to this drug of 100- to 1,000-fold (5). Moreover, 3TC selects for this mutation rapidly compared to the development of resistance to other drugs (16, 31, 35). The M184V mutation may also be selected on occasion by abacavir and didanosine (ddI), but it confers only low-level resistance to these compounds (11, 33), and in general, the presence of an M184V mutation alone, in the absence of other mutations, does not represent an obstacle to the use of either ddI or abacavir in antiviral chemotherapy. The M184V mutation is also associated with impaired viral fitness, increased RT fidelity, and hypersensitization to several other NRTIs (23). In this review, we discuss the various effects of the M184V mutation on viral fitness, delay of appearance of other mutations, and potential immunological consequences in HIV-infected individuals.

VIRAL FITNESS

Viral fitness is defined as the ability of a virus to adapt to its environment in terms of replicative capacity. Viruses with higher fitness can outcompete those of lower fitness as measured by tissue culture assays, including viral growth kinetics, single-cycle infection, and growth competition (26). The diminished fitness of viruses containing the M184V mutation has been shown both in vitro (3, 32) and in clinical studies (7, 9, 17, 20).

The estimated fitness of a given virus varies depending on the laboratory methodology, the viral strain utilized, and the type of cells used for culture; e.g., primary cells such as peripheral blood mononuclear cells, which contain low levels of deoxynucleoside triphosphate pools, may restrict viral replication more than cell lines which have higher deoxynucleoside triphosphate pools (3). HIV-1 RT processivity (which is defined as the number of nucleotides incorporated before the enzyme dissociates from the template) may be a major determinant of viral replication capacity or fitness (3, 6, 20, 32). It has been shown that defects in processivity, as measured biochemically, are often correlated with reduced replication fitness.

Clinical studies include the NUCA3001 study, in which patients who had received less than 4 weeks of zidovudine (ZDV) treatment were randomized to receive either 3TC monotherapy, ZDV monotherapy, or combination therapy with 3TC and ZDV for up to 52 weeks (9, 10). The results showed that viremia in the 3TC monotherapy arm attained a nadir of 1.2 $\log_{10}$ units by week 4, prior to viral load rebound, concomitant with the appearance of the M184V mutation; however, viremia consistently remained below baseline in patients who received 3TC and, moreover, was below that in patients who received ZDV alone. The AVANTI 2 and AVANTI 3 studies compared the efficacy of ZDV-3TC combined with a protease inhibitor, i.e., either indinavir (IDV) or nelfinavir (9). The development of the M184V mutation in both of these studies was associated with a significantly lower plasma viral load, while in contrast, the occurrence of ZDV-associated mutations had a negative effect on viral load. Finally, the Trilege trial was an induction-maintenance study in which patients began therapy with ZDV-3TC and IDV for 12 weeks, followed by maintenance therapy with either ZDV-3TC or ZDV-IDV for a further 6-week period. Removal of 3TC from the triple-drug regimen was associated with higher viral load at rebound compared to maintenance on ZDV-3TC (7), and the majority of patients on ZDV-3TC who experienced rebound harbored M184V.

EFFECT ON IMMUNE RESPONSE

The M184V mutation may also be associated with the ability of the immune system to suppress viral replication. Cytotoxic T lymphocytes (CTL) specific for HIV are considered to be a

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hallmark of efficient virus-specific immune responsiveness (4, 15, 21). The consequences of RT-associated mutations for recognition by CTL and the ability of host immune responsiveness to help control the growth of resistant variants has been evaluated in a limited way. In one study, a prospective analysis of CTL responses directed against RT mutations in patients treated with NRTIs was performed by using polyclonal HIV-specific CTL lines and evaluation of gamma interferon production (29). The M41L, L74V, and M184V mutations were all associated with greater CTL recognition than was an absence of mutations. In contrast, RT sequences in which the 215Y mutation was present were found to be poorly immunogenic. Another study showed specific CTL response to sequences that included the M184V mutation in only one patient out of 28 who were studied (30). It is possible that enhanced CTL recognition of some mutations may contribute to a lower replicative capacity of viruses that harbor these mutations. In addition, humoral immunity may play a role, and neutralization antibody titers from nine ZDV-treated subjects were shown to decline seven times faster than those in nine 3TC-treated patients (34). Another study showed a slower escape from neutralizing antibodies in HIV type 1 (HIV-1) variants containing M184V compared to wild-type virus, as a consequence of more limited variability in the envelope gene (12).

DELAYED APPEARANCE OF OTHER RESISTANCE-CONFERRING MUTATIONS

Resistance mutations for antiretroviral agents arise spontaneously as a result of the error-prone replication of HIV-1 and, in addition, are selected both in vitro and in vivo by pharmacological pressure (18, 25, 28). The high rate of spontaneous mutation in HIV-1 has been attributed largely to the absence of a 3′→5′ exonuclease proofreading mechanism. Sequence analyses of HIV-1 DNA have detected several types of mutations, including base substitutions, additions, and deletions (28). The frequency of spontaneous mutations for HIV-1 can vary considerably as a result of differences among viral strains (18). The overall mutation rates for wild-type laboratory strains of HIV-1 have been reported to range from 97×10^{-4} to 200×10^{-4} per nucleotide for HXB2 to as high as 800×10^{-4} per nucleotide for the HIV-1 NY5 strain (27, 28). This is, in part, a result of low RT fidelity. However, with a DNA template, M184V RT showed higher fidelity than did the wild-type enzyme (34); this may potentially affect the development of resistance to other antiretroviral agents. In the ALBI trial (19), for example, the T215Y mutation developed in a significantly higher proportion of patients who were randomized to treatment with ddI-stavudine (ddI-d4T) (62%) than in those who were treated with ZDV-3TC (10%) (24). The Q151M multinucleoside resistance mutation was also observed less frequently in patients who had been treated with 3TC (24). Similar results have also been reported in a retrospective analysis of the effect of the M184V substitution on the incidence of thymidine analogue-associated mutations (TAMs) and fold differences in phenotypic resistance to ZDV and d4T among isolates from treatment-experienced patients enrolled in the CNAB 3002 study. The results showed that the presence of M184V was associated with a significantly lower incidence of TAMs, notably D67N, L210W, and T215Y/F; moreover, this was independent of the plasma HIV-1 RNA level and duration of prior treatment with antiretroviral agents. Levels of phenotypic resistance to ZDV and d4T were also reduced in those patients in whom M184V was selected as a result of previous exposure to 3TC compared to patients in whom this mutation was not present (2).

The development of ZDV resistance was also evaluated in patients experiencing virological failure with 3TC-containing regimens in the AVANTI 2 and 3 clinical studies. In these trials, antiretroviral therapy-naive patients with HIV infection were randomly assigned to treatment with either 3TC-ZDV or 3TC-ZDV-IDV for 52 weeks in AVANTI 2 or with 3TC-ZDV-nelfinavir for 28 weeks in AVANTI 3 (17). Using combined data from both trials, genotypic analysis revealed ZDV resistance-conferring mutations in 27% of patients from the 3TC-ZDV arm of AVANTI 2, whereas these mutations were absent in patients from both arms of AVANTI 3 as well as in patients who received 3TC-ZDV-IDV in AVANTI 2. The M184V mutation, in these studies, was present in viral isolates from most patients who were treated with 3TC-ZDV. Overall, these results compare favorably to those from the CNA3003 study of abacavir intensification, in which selection rates for TAMs and Q151M were also reduced following the appearance of M184V (1). In regard to mutations associated with protease inhibitors and nonnucleoside reverse transcriptase inhibitors, a cell culture phenotypic assay study has shown that selection of resistance to efavirenz and amprenavir was delayed when viruses harbored M184V compared to the case for wild-type virus (8). Other studies have demonstrated that the presence of M184V may not significantly restrict the extent of mutagenesis in the protease gene (13, 14).

CONCLUSION

3TC was one of the first drugs shown to be associated with diminished HIV morbidity and mortality. Its benefit may be exerted even after emergence of M184V, a mutation that confers a high level of resistance to this drug. As briefly reviewed here, several mechanisms may be invoked to explain the clinical benefits associated with emergence of the M184V substitution in RT; these include decreased RT processivity, the possibility of enhanced immune responsiveness, increased RT fidelity, and diminished replicative fitness.

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