CONCISE COMMUNICATION

Human Immunodeficiency Virus–Infected Persons with Mutations Conferring Resistance to Zidovudine Show Reduced Virologic Responses to Hydroxyurea and Stavudine-Lamivudine

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The baseline predictors of poor virologic response (<0.5 log decrease in plasma virus load) were examined in two 1996 pilot trials of combination nucleoside-analogue therapy. One trial examined the addition of hydroxyurea to didanosine therapy; the other examined stavudine-lamivudine in combination. In both, predictors of virologic response included the presence of mutations associated with zidovudine resistance. For hydroxyurea, the odds ratio (OR) of failure to achieve a short-term (4 weeks) virologic response in a bivariate logistic regression model was 30.8 (95% confidence interval [CI], 1.75–543; P = .02) for using lower dose hydroxyurea (500 mg/day) and 14.7 (95% CI, 1.1–200; P = .04) for the presence of a zidovudine-related mutation. For the stavudine-lamivudine study, the OR of failure to achieve a virologic response at 4 weeks in a multivariate logistic regression model was 23 (95% CI, 2.7–199; P = .004) for the presence of a mutation at codon 215.

We previously reported results of two pilot studies designed to determine the short-term effect of hydroxyurea as an adjunct to didanosine monotherapy [1] and of stavudine-lamivudine combination therapy [2] in patients who were zidovudine intolerant or who no longer benefited from zidovudine therapy. Since prior exposure to nucleoside analogues may have minimized therapeutic effectiveness of these treatments, we wished to assess whether preexisting nucleoside-analogue resistance or other baseline parameters could account for the lack of virologic response to therapy in many persons in these pilot studies.

Subjects and Methods

Hydroxyurea study: subjects and study design. As reported elsewhere [1], human immunodeficiency virus (HIV)–infected persons with 100–350 CD4 cells and a minimum of 3 months of continuous didanosine therapy (n = 26) were enrolled in a 12-week open-label study to determine the antiviral effect of two doses of hydroxyurea as an adjunct to didanosine monotherapy. Eligible persons received didanosine monotherapy for the initial 4 weeks of the study (baseline phase) and then were randomly allocated to receive either 500 or 1000 mg/day of hydroxyurea plus continued didanosine for the next 4 weeks (hydroxyurea phase), followed by a return to didanosine monotherapy (washout phase). Plasma virus load (PVL) was measured weekly by Amplicor HIV-1 Monitor Assay (Roche Molecular Systems, Mississauga, Canada).

Virologic testing: DNA isolation and sequence. For the hydroxyurea study, baseline peripheral blood mononuclear cells were extracted by Isoquick kit (Orca Research, Bothell, WA) and the viral reverse transcriptase (RT) was amplified by nested polymerase chain reaction (PCR) with primers NE1 [3] and SC2960F (5′-CCATTAGTCTTATGAAACTGCCGAC-3′) in the first round and primers NV210R (5′-ACAGTCCAGCTGTCTTTTCTGGCAGC-3′) and SC2950F (5′-CCAAAGTAAAACATGGCATTGACAGA-3′) in the nested phase. The conditions and thermoprofiles used are described elsewhere [3]. Subsequently, both strands of the amplified DNA were sequenced from amino acid (aa) residues 31–236 in both directions by use of the second-round primers, SC225F (5′-ATTAGATATCAGTAATGCTGCTTCC-3′), or SC2930R (5′-GCATCAACCCATCCAGTACGTTGCT-3′), with use of ABI dye terminator sequencing kits (Applied Biosystems, Foster City, CA).

Stavudine-lamivudine study: subjects and study design. As reported elsewhere [2], 48 patients who could not tolerate zidovudine or who had zidovudine-therapy failure were treated with stavudine plus lamivudine in standard doses in an open-label study. PVLs were assessed at baseline and at weeks 2, 4, and 8 by the standard Amplicor HIV-1 Monitor Assay. Plasma viral nucleic acid was extracted by use of guanidinium isothiocyanate and isopropanol ethanol by standard techniques. Extracted HIV RT was amplified by nested RT-PCR using primers A-35 and NE-1 in the first round and primers comb2 and comb3 in the nested round. PCR conditions used were as reported elsewhere [3]. Both strands of amplified DNA were subsequently sequenced in both the 3′ and 5′ directions by use of primers BR (5′-GTTGATCCTTTCATCC-3′) and RH14...
(5'-TACTGCATTACCATACCTGATAAAC-3'), with the PRISM FS dye terminator cycle sequencing kit, and were resolved on an ABI 373 DNA sequencer (both Applied Biosystems).

Statistical methods. Virologic “response” was a 0.5 log decrease in virus load at week 4 compared with baseline, consistent with contemporary guidelines [4]. For the hydroxyurea study, the virus load was calculated as the average of the log$_{10}$ virus load measurements over weeks 1–4 (baseline) and weeks 5–8 (hydroxyurea). Subjects with >0.5 log$_{10}$ decrease in PVL from the baseline phase to the hydroxyurea phase were defined as having had a treatment response. Logistic regression was used to assess predictors (i.e., baseline CD4 cell count, baseline PVL, preexposure to zidovudine, didanosine, stavudine, or lamivudine, and AIDS-defining illnesses) of treatment response. No attempt was made to control for adherence. Baseline characteristics of responders and nonresponders were compared by use of Wilcoxon rank sum tests for continuous variables.

Results

Hydroxyurea. Of the 26 patients in the hydroxyurea study, 15 had mutations previously identified as being associated with resistance to zidovudine or didanosine (HIV RT codons M41L, D67N, K70R, L210W, T215Y/F/Q, or K219E for zidovudine; K65R, L74V, V75T, or M184V for didanosine). Thirteen subjects had 1 or more zidovudine-associated mutations (2 with M41L mutations; 2 with K70R; 1 with T215F/Y; 4 with M41L+215F/Y; 2 with D67N+K70R; 1 with M41L+D67N+K70R+T215F/Y; 1 with M41L+D67N+K70R+215F/Y+K219E), whereas 1 subject had a didanosine-associated mutation (K65R), and 1 had 2 didanosine-associated and 2 zidovudine-associated mutations (M41L+L74V+M184V+T215F/Y).

Eight subjects (1 assigned to 500 mg/day hydroxyurea and 7 assigned to 1000 mg/day hydroxyurea) met the criteria for treatment response. Baseline PVL of responders and nonresponders were similar (median, 3.7 log HIV RNA copies/mL, IQR, 3.3–4.5 log) for nonresponders; responders were similar (median, 3.3 log HIV RNA copies/mL, IQR, 2.6–4.2 log). Baseline CD4 cell counts were also similar for responders and nonresponders (median, 216 cells/mm$^3$, IQR, 153–299). Baseline characteristics of responders and nonresponders were compared by use of Wilcoxon rank sum tests for continuous variables.

Predictors of response were tested by use of logistic models (table 1). In a univariate analysis, subjects receiving the higher dose of hydroxyurea were 14 times more likely to have a treatment response than those receiving the lower dose ($P = .03$). When the model was run with a dose indicator, subjects with 1 or more mutations designated “zidovudine-specific” were 14-fold less likely to have a treatment response ($P = .04$). There remained a trend toward subjects with longer prior zidovudine exposure being less likely to have a treatment response, but this was not statistically significant ($P = .09$). There were no significant relationships between the probability of treatment response and prior duration of exposure to didanosine, baseline PVL, or baseline CD4 cell count (models 5–7, table 1). Because of limited numbers, we were unable to fit logistic regression models to test the relationship between treatment response and specific mutations. The 14 subjects with 1 or more zidovudine-specific mutations were evenly distributed with respect to the dose of hydroxyurea received but had longer prior zidovudine exposure (median, 2.7 vs. 0.6 years; $P < .001$) and higher PVL at baseline (median, 4.4 vs. 3.2 log HIV RNA copies/mL; $P = .04$) than those who exhibited no mutations. CD4 cell count and previous didanosine exposure were not statistically different between those with mutations and those without ($P > .14$).

Stavudine-lamivudine study. Of the 48 study participants, baseline resistance data was available for 46 (1 of whom was not observed at week 4, so no response data are available). Mutations of interest were identified at HIV-RT codons 41 (n = 11), 67 (n = 10), 69 (n = 8, including insertions at this codon), 70 (n = 11), 75 (n = 11), 184 (n = 15), 210 (n = 7), and 215 (n = 17). No aa substitutions were observed at codons 74, 75, or 151.

Univariate logistic regression models were fitted to determine the relationship between mutations at specific codons and PVL
response (data not shown). Mutations at codons 41, 184, and 215 had the strongest relationship with PVL response \( (P = 0.003, 0.007, \text{ and } 0.009, \text{ respectively}) \). Multivariate logistic regression models were used to further explore the relationship between resistance and virologic response (table 2). A mutation at codon 215 remained a significant predictor when other previously identified prognostic factors (e.g., baseline CD4 cell count, prior exposure to lamivudine or stavudine, or prior diagnosis of AIDS) were included in the model. Of interest, inclusion of the presence of a mutation of codon 184 in the model fully excluded the effects of prior exposure to lamivudine (table 2).

To investigate whether more than one mutation was an independent predictor of response, all mutations were allowed to enter a forward stepwise logistic regression model. Only the mutation at codon 215 entered the model (with an entry criteria of \( \alpha = 0.05 \)). When the entry criteria were increased to \( \alpha = 0.15 \), the mutation at codon 184 also entered the model, with a final odds ratio of 0.08 for the 215 mutation \( (P = 0.006) \) and 0.27 for the 184 mutation \( (P = .13) \). Patients with baseline plasma virus with the combination of mutations at codons 215 and 184 were particularly likely not to respond to stavudine-lamivudine.

Discussion

At least 6 aa changes in the HIV-1 RT (at codons 41, 67, 70, 210, 215, and/or 219) have been associated with resistance to zidovudine [5]. The results shown here suggest that preexisting mutations conferring resistance to zidovudine also predict failure with didanosine/hydroxyurea therapy and therapy with stavudine plus lamivudine, despite the fact that zidovudine was not included in these regimens.

In vitro and in vivo studies suggest that hydroxyurea, an inhibitor of the cellular enzyme ribonucleotide reductase, may enhance the antiretroviral effect of didanosine [6]. This effect seems to rely on an alteration of levels of the intracellular pools of dNTPs (especially dATP and ddATP), the active metabolite of didanosine [6]. Whereas this mechanism has been proposed to “control” drug resistance, the combination of hydroxyurea with didanosine does not prevent the selection of mutations associated with didanosine resistance [7]. More recently, hydroxyurea was shown to potentiate other nucleoside and nucleotide analogues against drug-resistant and -sensitive isolates in vitro [8]. Whereas preexisting zidovudine resistance is a poor prognostic marker for response to didanosine alone or in combination with zidovudine (see [9]), this effect has not been reported when didanosine is enhanced by coadministration of hydroxyurea. The dependence of the effect of hydroxyurea on mutations in the HIV RT supports the hypothesis that the mechanism of action of hydroxyurea is related to nucleoside metabolism rather than to its effects on the number of available target cells or other mechanisms [10].

At present, stavudine and lamivudine in combination are common components of HIV therapy. Previous studies have shown that preexposure to zidovudine reduces the efficacy of this regime [11]. Although the genetic basis of resistance to lamivudine has been well characterized, the same can not be said for stavudine. Mutations involved in stavudine resistance in vivo [12–15] have been difficult to define and may at least partially overlap with those selected by zidovudine. The predictive strength of mutations at codon 215 in this instance may reflect this overlap. Although phenotypic measures of resistance may be illuminating, they were not performed in this study.

In summary, our results demonstrate that mutations conferring resistance to zidovudine, specifically at codon 215, are likely associated with a diminished virologic response to regimens adding hydroxyurea to didanosine or regimens of stavudine plus lamivudine. These findings should be taken into consideration for patients with virologic failure associated with the development of nucleoside-analogue resistance.

References


