Antiviral Activity of the Human Immunodeficiency Virus Type 1–Specific Nonnucleoside Reverse Transcriptase Inhibitor HBY 097 Alone and in Combination with Zidovudine in a Phase II Study

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The safety and antiviral activity of the second-generation nonnucleoside inhibitor HBY 097 was investigated in asymptomatic or mildly symptomatic human immunodeficiency virus (HIV)-1–infected patients in a randomized, double-blinded, dose-escalation study. Mean maximum virus load decreases ranged from −1.31 log$_{10}$ copies/mL of plasma at week 1 in the group receiving HBY 097 monotherapy (250 mg three times daily) to −2.19 log$_{10}$ copies/mL at week 4 in the group receiving zidovudine plus HBY 097 (750 mg three times daily). After 12 weeks, these patients had viral RNA copy numbers 1.05 log$_{10}$ below baseline. Genotypic analysis of resistance development revealed reverse transcriptase K103N variants in most patients, which was associated with less durable efficacy of HBY 097 treatment. Fewer patients receiving combination therapy with high-dose HBY 097 developed the K103N variant ($P < .01$). HBY 097 caused pronounced acute suppression of HIV-1 replication both in combination with zidovudine and alone. Therefore, sustained antiviral activity can be expected from multiple combination therapy regimens including a quinoxaline derivative.

Certain quinoxaline derivatives have been described previously as highly potent inhibitors of human immunodeficiency virus type 1 (HIV-1) replication [1, 2]. These compounds act as nonnucleoside inhibitors of the viral reverse transcriptase (NNRTIs), and like other NNRTIs, quinoxalines are specific for the inhibition of the HIV-1 polymerase. IC$_{50}$s of selected compounds of the series were found to be in the low nanomolar range when tested against HIV-1 strains belonging to different subtypes [1, 2]. In contrast to other members of the NNRTI family, quinoxalines were shown to exert high selective pressure on the HIV-1 gene encoding the reverse transcriptase (RT), as characterized by the in vitro appearance of resistant enzyme mutants with crippled polymerase functions. G190E RT mutants have been shown to appear frequently with this class of NNRTIs during in vitro dose-escalation experiments [1–4]. HBY 097 was selected as a candidate for clinical development and proved its safety and potency in first dose-escalating phase I studies [5].

We describe here the antiviral efficacy of HBY 097 as a single drug and in combination with zidovudine during a multicenter, double-blinded, dose-escalation phase II clinical study. Variables measured include changes in plasma RNA virus load and the appearance of resistance-conferring RT gene mutations. The development of HIV-1 resistance was monitored by genotyping the polymerase domain of the RT gene from viral plasma RNA and also by sequence determination of proviral DNA obtained from peripheral blood leukocytes at different time points.

Methods

Patients. Study enrollment for protocol HBY 097/2001 started in the third quarter of 1995 at six different centers. The main objective of the study was determination of the safety and antiretroviral activity of multiple doses of HBY 097 alone and in combination with zidovudine. Eligibility criteria for participation in the trial were the presence of asymptomatic or mildly symptomatic HIV-1 infection with CD4 cell counts between 200 and 500 cells/µL, ≤ 4 weeks of prior antiretroviral therapy, and a minimum of 10,000 HIV-1 RNA genome equivalents/mL of plasma at two consecutive time points before initiation of treatment. Study subjects were at least 13 years of age and included males and nonpregnant and nonlactating females. Patients in each cohort were randomized in a 1:1 fashion to receive either HBY 097 plus zidovudine or HBY 097 plus zidovudine placebo for 12 weeks. Dosages of 250 mg and 750 mg of HBY 097 three times daily were chosen from a dose-escalation phase I study.

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ranging study [5]. During the following open-label phase study, participants were offered the HBY 097 plus zidovudine combination at the HBY 097 dosage administered during the previous 12 weeks.

Determination of virus load. This was done by use of the Amplicor HIV monitor assay for quantification of viral RNA (detection limit, 200 HIV-1 RNA copies/mL of plasma), according to the instructions of the manufacturer (Roche Molecular Systems, Nutley, NJ).

Proximal RT gene sequences. EDTA-anticoagulated blood was used at each sampling date for purification of DNA from total peripheral blood leukocytes. Nested primerase chain reaction (PCR) was done as described, and the analysis of sequencing reactions (RT codons 35–242) was done with an ALF automated DNA sequencer (Pharmacia, Freiburg, Germany) [3].

Sequences from plasma-derived pol genes. Plasma RNA was isolated, reverse-transcribed, and PCR-amplified according to previously described procedures [6].

A second round of PCR amplification was done by use of primers MAW12-T3 (5'-AATTAACCTCATAAGGAGATGCTAC-CAGTAAAAATAAG) and MAW13-T7 (5'-TTATACGACT-CATAAGGAGATGTCTTTCTGTTAGCATA). Fluorescent RNA was then transcribed from the second-round PCR products by use of either T3 or T7 RNA polymerase. The fluorescent RNA was fragmented by heating at 95°C for 30 min. The sequence of the RNA was then analyzed by use of Gene-Chip high-density oligonucleotide arrays (Affymetrix, Santa Clara, CA), according to the manufacturer’s instructions.

Results

Patient population, study design, and safety. In the first cohort, 30 patients were randomized to receive either 250 mg of HBY 097 three times daily plus zidovudine placebo or 250 mg of HBY 097 plus 200 mg of zidovudine three times daily for 12 weeks. After completion of this double-blind phase, all subjects were allowed to continue with the zidovudine plus HBY 097 combination as part of an open-label extension. For a second cohort, an equal number of patients was randomized, and the HBY 097 dosage was raised to 750 mg three times daily. Mean ages were 31.6 and 34.4 years for subjects in the 250 mg and 750 mg cohorts, respectively. In each group, 87% of the patients were male and 13% were female. Median CD4 cell numbers were 334 and 401 cells/μL in the low- and high-dose groups, respectively, and the baseline virus load in these groups was 4.66 and 4.59 log10 copies/mL, respectively.

The study medication was generally well-tolerated. The major adverse event observed during the treatment period was rash (total incidence, 12%), followed by nausea, headache, and neutropenia with anemia, the latter to be related to zidovudine. Sixteen patients discontinued because of adverse events before completion of the double-blinded phase, 3 patients were lost to follow-up, and 2 subjects discontinued for other reasons. Twenty-eight patients participated in the open-label extension and received HBY 097 plus zidovudine.

Changes in plasma virus load. The course of virus load by treatment group is shown in figure 1. Mean maximum reductions in viral RNA/mL of plasma of −1.31 log10 (at week 1) and −1.90 log10 (at week 2) were observed in those receiving HBY 097 monotherapy at 250 mg and 750 mg, respectively. There was a maximum decrease of −1.90 log10 (at week 2) in those receiving combination therapy with HBY 097 at 250 mg. The highest decline in plasma viral RNA was noted in the combination arm at 750 mg of HBY 097, in which a mean maximum drop of −2.19 log10 in RNA copy numbers occurred at week 4. After 12 weeks of therapy, viral RNA remained −1.05 log10 below baseline in this patient group, which compares to −0.54 log10 in the group receiving low-dose HBY 097 with zidovudine. In the monotherapy arm, mean RNA levels had returned to baseline after 12 weeks (−0.10 log10 at 250 mg and +0.26 log10 at 750 mg).

Evaluation of genotypic resistance development. Some patients displayed drug resistance–associated mutations in their proviral RT genes before the initiation of treatment (NNRTI-related: A98S,2; K103R,2; V106I,2; V179D,1; V189I,1; nucleoside RT inhibitor–related: M41L,1; T69N,1; K70R,2; T215N/S,1; T215Y,1). Some of these mutations can be considered natural variations; however, the presence of such genotypes as K70R and T215Y suggests previous treatment of these patients with zidovudine. Genotypic changes during the treatment period were determined at both the proviral and plasma viral RNA level. The G190E mutant, which was frequently observed in vitro with HBY 097, did not show up during the course of this study. The most abundant mutation detected during this study caused a K103N RT amino acid substitution. This change was seen in 31 (78%) of 40 patients who were followed up for at least 12 weeks (figure 2). The proportion of patients with viruses developing K103N was similar in 3 of the 4 treatment groups. Interestingly, 2 of the 3 patients in these groups who did not show a K103N mutant had other NNRTI-specific mutations already present at baseline: In one case, an Arg at position 103 was replaced by a Ser during treatment, and in the other, a V106A change was added to an existing V179D background.

There was a significantly greater number of patients without the predominant K103N mutation in the group receiving high-dose HBY 097 in combination with zidovudine than in the other 3 groups (6/11 vs. 1/9 in each of the monotherapy groups and 1/11 in the low-dose combination group; P < .01, χ2 test). Within the high-dose HBY 097 combination therapy group, 4 of the 6 subjects without a K103N change did not show any other resistance mutation. NNRTI-related mutations selected during the study are as follows (in descending frequency): K103N,31; Y181C,5; G190A,3; K101I,2; K101V,2; Y188L,2; M230L,2; A98S,1; K101E,1; K101T,1; K103S,1; V106A,1; Y188H,1; V189I,1; P225H,1. There were no nucleoside RT inhibitor–related mutations selected during this study. Among the deviations that
were detected in the patient population and not considered to be associated with administration of the study medication, aa 135 showed the greatest variability (I135G, L, M, T, or V).

Discussion

HBY 097 showed pronounced antiviral activity, both alone and in combination with a single nucleoside RT inhibitor (zidovudine), during this phase IIa study. The maximum degree of suppression of viral replication was achieved in the group receiving 750 mg of HBY 097 three times daily plus zidovudine, with respect to both mean maximum decrease in plasma virus load (−2.19 log10 HIV RNA copies/mL) and duration of the response (figures 1, 2). One patient in this group still showed undetectable plasma viral RNA at week 20.

Viruses with NNRTI-specific resistance mutations were detected in most patients (35/40, 88%), and the appearance of these mutations was associated with an increase in plasma viral RNA copy numbers. The main substitution seen was an RT K103N change, as previously observed with other NNRTIs [7]. Although we have reported the occasional in vitro occurrence of K103N multiple mutants, the predominance of this mutation in vivo was not expected from these dose-escalating experiments.

In this study, the development of K103N was not influenced by the preexistence of zidovudine resistance genotypes or by the combination of low-dose HBY 097 with zidovudine (figure 2). In contrast, there was a statistically significant correlation between the lower frequency of appearance of the codon 103 mutant and both high-dose HBY 097 and coadministration of zidovudine.

The impact of the K103N mutation on HBY 097 activity was investigated by various methods. Using HBY 097, Hsiou et al. [8] could attain the formation of crystals of a purified K103N mutant enzyme and were able to resolve the three-dimensional structure of this RT-inhibitor complex at 2.8 Å.
resolution, showing that HBY 097 still binds with high affinity to the same site on the altered protein. Virus inhibition assays in cell culture using 1 wild type strain and 2 different corresponding K103N mutant variants resulted in an average 19.5-fold reduction in HBY 097 activity. EC50s were in the nanomolar range in all experiments, showing that replication of HIV-1 strains with a K103N mutated RT gene can be easily suppressed in vitro (data not shown). These data were also confirmed by enzyme assays. It could be shown that the inhibitory activity of HBY 097 against a K103N-mutated RT was only 13-fold lower than that of the control protein without the substitution at position 103 [9]. IC50s measured with mutant enzymes obtained by insertion of K103N RT gene fragments from patients of this study into a RT cassette vector did indicate ~10-fold reduced susceptibility of these mutants to HBY 097 (Dunkler A, unpublished data).

Therefore, since the presence of a K103N RT does not lead to inactivation of HBY 097 in vitro, we conclude that the pharmacokinetic parameters involved play a critical role in the repeated in vivo selection of the K103N genotype under conditions existing in the present study. In other words, the concentration of HBY 097 at the sites of viral replication in vivo was not sufficient to suppress replication of a virus variant that displays only a limited decrease in susceptibility. In support of this hypothesis is the fact that a higher dose of HBY 097 did reduce the frequency of K103N variants when zidovudine was coadministered.

The particular predominance of the K103N RT remains puzzling, since other single mutants do confer a similar degree of low-level resistance against HBY 097 in vitro [2]. Virus populations present in untreated patients have been shown to harbor genotypes conferring drug resistance to both protease and RT inhibitors [10]. Possibly, a higher portion of K103N RT genes preexists in these treatment-naive patients compared with genomes containing the other substitutions, such as V106A, Y181C, or G190A, thereby explaining the findings reported here.

Although escape variants described in this study did contain the K103N single mutation in most cases, some patients developed multiple mutants (figure 2 and data not shown). Of note, K103N was always the first change to show up, before either Y181C (5 patients), Y188H (1 patient), or G190A (3 patients) mutations were added to the single mutant genotypes (figure 2). The finding of these multiple mutants with HBY 097 in this study resembles the in vitro simulation of low selective pressure conditions with the same drug [4].

The exact role of the recently described P225H substitution remains to be clarified. In vitro, this mutation appeared only in an RT V106A background with both quinoxaline S-2720 [11] and HBY 097 [4]. P225H did not confer resistance to these compounds as a single mutation in recombinant RT constructs, but it did contribute to a less sensitive phenotype in V106A/P225H mutant viruses [11]. In contrast, the patient who showed the P225H substitution in this study had already developed a K103N RT, and the K103N/P225H double mutant was detected for the first time after 25 weeks of low-dose HBY 097 in combination with zidovudine treatment (figure 2).

The antiviral activity exerted by HBY 097 in the present study compares well with data from similar trials including NNRTIs either as single agents or in combination with a nucleoside analogue [12–14]. Since multiple drug combinations including first-generation NNRTIs have been shown to result
in more prolonged suppressive effects on HIV replication [15], the data presented here support the concept of therapy regimens including a quinoxaline-class NNRTI. However, some other members of this large series of compounds were found recently to offer a greater possibility than HBY 097 to meet the criteria for a second-generation NNRTI, such as high potency, once-daily dosing, good tolerability, significant central nervous system penetration, and few drug interactions.

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References