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Drug Sensitivity of Human Immunodeficiency Virus Type 1 Isolates after Ribavirin Therapy

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The antiviral agent ribavirin is effective against several virally induced diseases, and there is evidence that it might prove useful against human immunodeficiency virus type 1 (HIV-1) infection. Thus, there is interest in studying the resistance level of HIV-1 isolates to ribavirin following drug therapy. Low-passage clinical strains of HIV-1 were isolated from 3 patients undergoing treatment with ribavirin for 5–9 months. No significant changes in drug sensitivity were seen for sequential virus samples obtained before, during, and after antiviral therapy. These observations suggest that the appearance of a resistant phenotype is not induced by treatment with ribavirin in HIV-1-infected persons.

Ribavirin (1-β-d-ribafuranosyl-1,2,4-triazole-3-carboxamide) is a synthetic guanosine-like nucleoside that targets both RNA and DNA viruses [1]. This compound readily enters eukaryotic cells, where it is converted to ribavirin mono-, di-, and triphosphate forms by cellular enzymes. The latter step is crucial because the most active form of the drug is its triphosphorylated isoform [2].

Ribavirin’s broad antiviral spectrum includes activity against human immunodeficiency virus type 1 (HIV-1). Indeed, McCormick et al. [3] have reported that replication of HIV-1 in primary peripheral blood mononuclear cells (PBMC) was inhibited by 50–100 µg/mL ribavirin (205–410 nM). Further studies have shown that high concentrations (>6 mM) of ribavirin inhibit the HIV-1 reverse transcriptase (RT) enzyme [4].

Inconclusive data have been obtained in clinical trials of ribavirin monotherapy in HIV-1–infected patients [5, 6]. Moreover, as shown by Vogt et al. [7], ribavirin antagonizes the inhibitory effect of zidovudine on HIV-1 replication in infected cells. Vogt et al. postulated that ribavirin and zidovudine compete for the same cellular kinases, since both are highly active in their phosphorylated forms. However, in contrast to zidovudine, ribavirin enhances the antiretroviral activity of purine analogues, such as 2′-3′-dideoxyinosine (ddl) [8]. Furthermore, concentrations of ribavirin enhanced inhibition of HIV-1 replication by ddl and could even limit the appearance of ddl-resistant HIV-1 particles [9].

The emergence of HIV-1 particles possessing a drug resistance phenotype during HIV-1 antiviral therapy is frequent and has been well documented (reviewed in [10]). For example, in vitro zidovudine resistance occurs as early as 6 months after initiation of drug therapy [11]. The frequency of isolation of zidovudine-resistant strains of HIV-1 was reported to increase with duration of drug therapy and to be associated with advanced stages of disease [12].

The aim of the present study was to determine whether drug resistance is likely to occur in HIV-1–infected persons receiving ribavirin therapy. Sequential isolates of HIV-1 from 3 patients who received ribavirin therapy were evaluated for their drug susceptibility.

Methods

Study population. The 3 patients participating in the study were part of a double-blind placebo-controlled clinical trial comparing ribavirin with a placebo in 462 asymptomatic patients who had ≥300 but ≤800 CD4 cells/mm³ and who were p24 antigen negative and HIV-1 antibody positive [13]. These 3 asymptomatic patients were treated for 5–9 months with oral ribavirin (1200 mg/day, divided into three 400-mg doses) and were followed for up to 10 months following cessation of therapy.

Virus isolation and drug sensitivity test. PBMC from healthy seronegative donors were stimulated with phytohemagglutinin (3 µg/mL) for 48–72 h, washed, and resuspended in complete medium (RPMI 1640 with 30 U/mL recombinant interleukin-2, 10% fetal calf serum, 2 mM L-glutamine, 25 mM HEPES, 25 mM 2-mercaptoethanol, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate). Clinical HIV-1 isolates were obtained by coculturing 10⁶ PBMC from patients A, B, and C with 10⁶ phytohemagglutinin-stimulated normal donor PBMC. The cultures were incubated at 37°C in 5% CO₂. Culture medium was replaced every 3 or 4 days. Freshly stimulated donor PBMC (5 × 10⁶) were added at days 3,
Table 1. Patients’ profiles, sequential primary HIV-1 isolates, and susceptibility to ribavirin.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex, age (years)</th>
<th>CD4 cell count (/mm³)</th>
<th>Duration of ribavirin therapy (months)</th>
<th>Time after cessation of drug treatment (months)</th>
<th>Isolate</th>
<th>IC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>M, 39</td>
<td>774</td>
<td>0</td>
<td>—</td>
<td>35</td>
<td>51.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>706</td>
<td>3</td>
<td>—</td>
<td>108</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>543</td>
<td>6</td>
<td>—</td>
<td>140</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>761</td>
<td>—</td>
<td>6</td>
<td>311</td>
<td>2.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>781</td>
<td>—</td>
<td>10</td>
<td>367</td>
<td>88.8</td>
</tr>
<tr>
<td>B</td>
<td>M, 41</td>
<td>595</td>
<td>0</td>
<td>—</td>
<td>53</td>
<td>160.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>609</td>
<td>1</td>
<td>—</td>
<td>111</td>
<td>102.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>476</td>
<td>6</td>
<td>—</td>
<td>177</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>397</td>
<td>9</td>
<td>—</td>
<td>229</td>
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<tr>
<td></td>
<td></td>
<td>514</td>
<td>—</td>
<td>2</td>
<td>290</td>
<td>142.4</td>
</tr>
<tr>
<td>C</td>
<td>M, 45</td>
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<td>—</td>
<td>31</td>
<td>58.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>495</td>
<td>1</td>
<td>—</td>
<td>103</td>
<td>52.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>361</td>
<td>5</td>
<td>—</td>
<td>150</td>
<td>72.5</td>
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<tr>
<td></td>
<td></td>
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<td>1</td>
<td>220</td>
<td>47.7</td>
</tr>
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<td></td>
<td></td>
<td>295</td>
<td>—</td>
<td>2</td>
<td>230</td>
<td>25.8</td>
</tr>
</tbody>
</table>

NOTE. NT: not tested.

7, 14, and 21. Supernatants were then used to infect fresh PBMC, and viral replication was evaluated by an RT microassay. Aliquots of clarified supernatants were stored at −80°C after RT activity had reached >500,000 cpm/mL. These virus stocks were used in the ribavirin sensitivity assay.

The in vitro drug sensitivity assay was done in 96-well plates with 2 × 10⁵ PBMC from seronegative donors and 200 µL of culture medium/well in the presence of clinical strains of HIV-1 isolated from patients treated with ribavirin. In brief, PBMC were first preincubated at 37°C for 90 min with equivalent amounts of virus (25,000 cpm of RT activity/well). Ribavirin was next added at final concentrations of 0, 10, 25, 50, 100, and 250 mg/mL. For assessment of viral growth, the mean RT activity was measured 10 days after infection for each ribavirin concentration and expressed as counts per minute per 50 µL, based on the mean of three different wells. The IC₅₀ values for the susceptibility of virus growth to ribavirin were calculated with the computer effect equation. HIV-1₄₀, which was harvested from culture fluids of a chronically infected H9 cell line (provided by R.C. Gallo; NIH, Bethesda, MD), was used as a control.

**RT assay.** RT activity was assessed using a previously reported procedure as described [14], with minor modifications.

Results

The patient profile is depicted in table 1. Subjects included 3 men (39-45 years old) who had experienced a decrease in CD4 cell count during ribavirin treatment, although they maintained close to 400 CD4 cells/mm³. After treatment ended, the absolute CD4 T cell count continued to decline for patient C but increased for patients A and B. When viral culture was attempted with donor PBMC, the virus could be isolated from cryopreserved PBMC from subjects at five time points — before treatment, at 3–9 months during therapy, and at 2–10 months following the cessation of antiretroviral therapy.

To test whether ribavirin resistance was occurring in such treated patients, an in vitro drug sensitivity assay was done. PBMC isolated from healthy donors were infected with low-passage clinical strains of HIV-1 derived from the 3 study subjects in the presence of an expanded range of ribavirin (0–250 µg/mL). The IC₅₀ for sequential isolates from study patients are shown in table 1. The 3 pretherapy clinical virus isolates tested showed an IC₅₀ varying between 51 and 161 µg/mL. These values are consistent with the concentration of ribavirin necessary to inhibit 50% of the laboratory isolate HIV-1₄₀ (IC₅₀ of 93.7 µg/mL; data not shown). There was no appearance of resistance to ribavirin with increasing duration of therapy. Indeed, very little variation could be seen in the IC₅₀ after 5–9 months of therapy. Last, we analyzed the ribavirin susceptibility of isolates from the 3 study subjects, who had stopped drug therapy and not had alternative antiretroviral therapy. The 5 posttherapy isolates tested were not more sensitive to inhibition by ribavirin, with IC₅₀ ranging from 3 to 142 µg/mL.

These data thus suggest that virus particles from HIV-1–infected patients treated for different periods of time with ribavirin are still sensitive to the antiviral effect of ribavirin in vitro.

Discussion

We investigated the development of ribavirin resistance in 3 symptom-free persons undergoing drug therapy for 5–9 months. We analyzed the ribavirin susceptibility of 14 isolates of HIV-1 obtained before, during, and after treatment with the drug. Because zidovudine-resistant HIV-1 particles are rapidly and frequently generated following treatment of infected per-
sons with zidovudine [10–12], we were interested in seeing whether the same is also true for ribavirin.

Our results, based on sequential clinical isolates from 3 treated patients, showed no increase in resistance to ribavirin as monitored by IC{50} values, regardless of the length of ribavirin therapy or time after cessation of therapy. These data suggest that ribavirin is still a virustatic agent under physiologic conditions. These results are in agreement with in vitro studies demonstrating the inability to derive herpesvirus type 1 with a resistance phenotype to ribavirin [15].

Ribavirin is a highly soluble compound showing easy penetration of eukaryotic cells. This feature, coupled with our observations indicating that no ribavirin resistance is generated following drug therapy, makes this compound a good candidate for anti-HIV-1 therapy. In addition, because of its reported potentiating effect on the in vitro and in vivo antiviral activities of ddI [8], we believe that ribavirin should be considered as a potential drug in combination therapy with ddI. ddI and ribavirin act at different sites in the replicative cycle of HIV-1, and a combination of these agents may offer multiple advantages, including synergistic antiviral effects, more efficient suppression of viral replication, reduced doses of each drug below their respective toxic concentrations, and a lowered probability of selecting viruses with a drug-resistant phenotype.

Ribavirin should be perceived as an antiviral drug that can be used in combination therapy in the treatment of retrovirus infections. Ribavirin alone is generally well tolerated; however, there have been reported side effects, including anemia, when doses are too high or given for too long. Within a few weeks following therapy, ribavirin induces a minor reduction in red blood cells counts, neutrophils, and lymphocytes; the reductions peak at 4 weeks and do not, in general, increase further. These abnormalities usually subside as the drug is withdrawn. The combination of ribavirin with other antiretrovirals deserves consideration for the treatment of HIV-1–infected persons.

To our knowledge, our study is the first to demonstrate that ribavirin treatment does not lead to the emergence of clinical isolates of HIV-1 with reduced drug sensitivity.

Acknowledgments

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References