Effects of Formulation and Dosing Strategy on Amprenavir Concentrations in the Seminal Plasma of Human Immunodeficiency Virus Type 1–Infected Men

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We compared seminal plasma pharmacokinetic data for the investigational amprenavir prodrug GW433908 with those for amprenavir and an amprenavir-ritonavir combination regimen. All 3 regimens resulted in detectable blood plasma and seminal plasma concentrations of amprenavir. The majority of these concentrations were greater than the plasma protein–corrected 50% inhibitory concentration for wild-type human immunodeficiency virus type 1.

Published data have demonstrated that the male genital tract can serve as a distinct biological compartment and reservoir for viral replication [1, 2]. Elevated HIV-1 RNA concentrations in blood and seminal plasma have been associated with an increased transmission risk [3]. Therefore, the use of antiretroviral drugs that adequately penetrate the male genital tract could potentially affect transmission rates. Conversely, poor penetration of antiretrovirals into the male genital tract makes them inadequate to prevent viral replication, thereby increasing the risk of transmission and the emergence of drug-resistant variants, which may also be transmitted or may provide a source for systemic viral rebound [2].

Recent studies have evaluated the penetration of antiretroviral medications into the male genital tract [4]. Generally, the nucleoside-analogue reverse-transcriptase inhibitors zidovudine, lamivudine, and stavudine achieve seminal plasma concentrations equal to or greater than the concentrations in blood plasma. The nonnucleoside reverse-transcriptase inhibitors efavirenz and nevirapine generally achieve seminal plasma concentrations that are less than the blood plasma concentration but greater than the IC50 of wild-type virus. The protease inhibitors generally achieve the lowest concentrations in the male genital tract, although the ratios of semen to plasma concentration vary for each compound (the semen:plasma ratios are greatest for indinavir, followed by amprenavir, followed by ritonavir and saquinavir, followed by nelfinavir).

Amprenavir (Agenerase; Vertex Pharmaceuticals and GlaxoSmithKline) is a lipophilic protease inhibitor that is ~90% bound to plasma proteins [5]. Amprenavir achieves greater seminal plasma concentrations than do some other protease inhibitors, such as ritonavir and saquinavir [1, 6]. An effective viral response has been demonstrated in the seminal plasma of men treated with amprenavir as monotherapy [7].

APV20001 was a phase II clinical study involving treatment-naive HIV-1–infected male subjects that was designed to compare the pharmacokinetics of the investigational amprenavir prodrug GW433908 (Vertex Pharmaceuticals and GlaxoSmithKline) with those of amprenavir. This comparative crossover phase was followed by treatment with amprenavir-ritonavir (Agenerase and Norvir [Abbott Laboratories]) and additional study of pharmacokinetics. During this investigation, we evaluated the concentration of amprenavir in the seminal plasma of 2 men during each of the 3 treatment periods.

Written informed consent was obtained from all subjects. Human experimentation guidelines of the US Department of Health and Human Services and those of the University of North Carolina at Chapel Hill were followed in the conduct of this clinical research. Steady-state blood and semen samples were obtained during 3 study treatment phases: (1) while subjects were receiving amprenavir (1200 mg b.i.d.), (2) while they were receiving GW433908 (1200 mg or 1600 mg of amprenavir equivalents b.i.d.), and (3) while they were receiving amprenavir-ritonavir (amprenavir, 600 mg b.i.d; ritonavir, 100 mg b.i.d.). All subjects also received abacavir (Ziagen; GlaxoSmithKline; 300 mg b.i.d.) and lamivudine (Epivir; GlaxoSmithKline; 150 mg b.i.d.).
Blood and semen samples were centrifuged at 2500 g. Semen samples, which were removed and frozen at 20 min for blood and 10 min for semen), and the resulting plasma samples (concentrations measured on separate days 12 h after administration of the dose; NA, not applicable; Rtv, ritonavir.

During each study phase, which lasted 28–35 days, 3–5 semen samples (concentrations measured on separate days 12 h after receipt of the dose [C\(_{12h}\)]) and 14–16 blood samples were obtained under steady-state conditions. Semen samples, which were provided by masturbation, were left to liquefy for 30 min. Blood and semen samples were centrifuged at 2500 g (20 min for blood and 10 min for semen), and the resulting plasma was removed and frozen at –80°C until analyzed. Amprenavir concentrations were measured in blood plasma and seminal plasma samples by liquid chromatography with mass spectrometry using a validated assay [6]. Abacavir and lamivudine concentrations were not measured. The area under the concentration-time curve during the 12 h dosing interval (AUC\(_{12h}\)) was determined by noncompartmental methods using WinNonLin Pro V3.3 (Pharsight). HIV-1 RNA levels were measured in blood plasma and seminal plasma using the ultrasensitive Amplicor HIV-1 Monitor Test 1.0 (Roche Diagnostics) and Organon Teknika Nuclisens kits, respectively. The lower limits of detection for the Amplicor and Nuclisens kits are 50 copies/mL (1.7 log\(_{10}\) copies/mL) and 400 copies/mL (2.6 log\(_{10}\) copies/mL), respectively. Data are reported as geometric mean ± SD.

Two white men (age of subject 1, 44 years; age of subject 2, 36 years) were enrolled and completed this APV20001 substudy. Subject 1 had a baseline blood plasma HIV-1 RNA level of 4.6 log\(_{10}\) copies/mL. He received therapy for 20 weeks; at week 20, his blood plasma HIV RNA level was <1.7 log\(_{10}\) copies/mL. Subject 2 had a baseline blood plasma HIV-1 RNA level of 3.7 log\(_{10}\) copies/mL. Subject 2 received therapy for 16 weeks; at week 16, his blood plasma HIV-1 RNA level was 2.5 log\(_{10}\) copies/mL. For both subjects, the seminal plasma HIV-1 RNA levels at baseline were <2.6 log\(_{10}\) copies/mL, and the levels remained at less than this limit of detection for the course of the investigation.

The blood plasma AUC\(_{12h}\) and C\(_{12h}\) and the seminal plasma C\(_{12h}\) for both men were similar to those reported elsewhere (table 1) [6, 8]. During each phase of the study, intra-individual variability (measured as percent coefficient of variation) in blood plasma and seminal plasma concentrations of amprenavir had ranges of 8%–78% and 26%–76%, respectively. Intra-individual variability in ratios of seminal plasma to blood plasma concentrations had a range of 7%–62%. The GW433908 formulation did not alter genital tract exposure to amprenavir. Amprenavir-ritonavir (amprenavir, 600 mg b.i.d.; ritonavir, 100 mg b.i.d.) increased amprenavir blood plasma AUCs 1.6–1.9-fold and blood plasma C\(_{12h}\) 1.8–6.8-fold. Amprenavir seminal plasma C\(_{12h}\) increased 1.5–2.1-fold (table 1).

All 3 amprenavir regimens resulted in detectable blood plasma and seminal plasma concentrations of amprenavir. The majority of these concentrations were greater than the plasma protein–corrected IC\(_{50}\) for wild-type virus reported by 2 different methods: (1) wild-type (IIIb) HIV-1 isolates cultured in

### Table 1. Steady-state pharmacokinetic parameters of amprenavir (Apv) in the blood and seminal plasma of 2 HIV-1–infected men.

<table>
<thead>
<tr>
<th>Measurement, by regimen (no. of measurements)</th>
<th>GW433908</th>
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<tbody>
<tr>
<td>Subject, parameter</td>
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<tr>
<td>Blood plasma AUC(_{12h}), µg/mL</td>
<td></td>
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<tr>
<td>Blood plasma C(_{12h}), µg/mL</td>
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<td>Ratio of seminal plasma concentration to blood plasma concentration</td>
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<tr>
<td>Blood plasma vitamin E, µM(^a)</td>
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<td>Seminal plasma C(_{12h}), µg/mL</td>
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<td>Blood plasma C(_{12h}), µg/mL</td>
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<td>Blood plasma vitamin E, µM(^b)</td>
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| Highlights                                                                 |          |
|---                                                                         |          |
| Data are mean ± SD, unless otherwise indicated. Baseline vitamin E concentrations were obtained before the administration of medication on day 1 of study enrollment. AUC\(_{12h}\), area under the concentration-time curve during the 12-h dosing interval; C\(_{12h}\), concentration obtained 12 h after administration of the dose; NA, not applicable; Rtv, ritonavir.

\(^a\) Baseline value, 15.3 µM.

\(^b\) Baseline value, 25.8 µM.

**NOTE.**
50% human serum (IC_{50}, 0.147 ± 0.051 µg/mL) [9], and (2) mathematical correction of the IC_{50} of 334 clinical isolates for 90% protein binding (IC_{50} 0.146 ± 0.125 µg/mL) [10]. The mean protein-corrected ratios of C_{12h} to IC_{50} had ranges of 1.4–11.2 in blood plasma and 0.75–2.3 in seminal plasma (although the degree of protein binding of any antiretroviral agent in the genital tract is currently unknown).

As has been suggested with cyclosporine [11], the soluble vitamin E present in amprenavir capsules has been implicated in inhibiting the P-glycoprotein–mediated efflux of amprenavir [12] in addition to increasing amprenavir solubility. Because GW433908 contains no vitamin E, this formulation hypothetically may result in lower concentrations of amprenavir in the genital tract through less P-glycoprotein inhibition, resulting in greater amprenavir efflux from that compartment. However, the GW433908 formulation achieved seminal plasma concentrations that were similar to those achieved by the amprenavir formulation, despite there being elevated vitamin E concentrations in the blood plasma of both subjects while they were receiving amprenavir therapy (table 1). These findings support recent in vitro data that demonstrate that vitamin E alone is not a cytochrome P450 3A subfamily or P-glycoprotein inhibitor [13].

Recently, Van Praag et al. [14] described 6 patients who were receiving indinavir (1000 mg t.i.d.) as part of their antiretroviral regimen and whose regimens were switched to indinavir-ritonavir (indinavir, 800 mg b.i.d.; ritonavir, 100 mg b.i.d.). The addition of ritonavir to the treatment regimen increased the median minimum concentration of indinavir in blood plasma from 65 ng/mL to 336 ng/mL (P = .005) and the median seminal plasma concentrations from 141 ng/mL to 1634 ng/mL (P = .002). The median 8.2-fold increase in the seminal plasma concentration of indinavir (95% CI, 5.2–11.6) could not be explained by greater indinavir serum trough concentrations alone, which suggests that ritonavir inhibition of cellular efflux transporters decreased indinavir efflux from the male genital tract.

For the 2 subjects described in this report, the addition of ritonavir to treatment did not disproportionately increase the seminal plasma concentration of amprenavir. Polli et al. [15] published similar findings about the role of ritonavir in the disposition of amprenavir in the CNS. Although mdr1a/1b double-knockout mice and chemical-knockout mice (pretreated with the P-glycoprotein inhibitor GF120918) had a 3.3-fold increase in amprenavir concentrations in CSF, ritonavir did not alter CNS exposure to amprenavir [15]. These data suggest that amprenavir concentrations at sequestered sites (e.g., the genital tract and CNS) may not be highly dependent on ritonavir-inhibited cellular efflux transporters.

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References