Stable Concentrations of Zidovudine, Stavudine, Lamivudine, Abacavir, and Nevirapine in Serum and Cerebrospinal Fluid during 2 Years of Therapy

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For a number of antiretroviral drugs, prolonged suppression of viral replication is related to drug exposure. Therefore, it is important to maintain stable concentrations during prolonged therapy. While studies suggest that saquinavir concentrations decrease over time, we show that concentrations of zidovudine, stavudine, lamivudine, abacavir, and nevirapine in serum and cerebrospinal fluid are stable during 2 years of therapy.

With the advent of currently available combination antiretroviral therapy, 50 to 80% of human immunodeficiency virus type 1 (HIV-1)-infected patients maintain undetectable plasma HIV-1 RNA levels after 1 year of therapy (5, 12). However, the percentage of patients with plasma HIV-1 RNA levels below 50 copies/ml decreased from 75% after 1 year to 65% after 3 years of triple therapy (5). It is not clear how this late virological breakthrough in patients on stable antiretroviral therapy should be explained. One possible explanation might be suboptimal drug concentrations, due to decreased adherence to antiretroviral therapy over time or due to a gradual decrease in antiretroviral drug concentrations over time despite good adherence (4, 8). For instance, concentrations of the protease inhibitor (PI) saquinavir in plasma have been shown to decrease substantially over time (4, 8). As the exposure to PIs has been linked to virological success and toxicity, regular monitoring of PI concentrations, even in apparently compliant patients, is suggested to become part of routine patient care (2). The exposure to nonnucleoside reverse transcriptase inhibitors (non-NRTIs) has also been linked to efficacy and toxicity (6, 9, 17). Recent clinical trials showed that for nevirapine (NVP), steady-state concentrations above 3.4 μg/ml are necessary for long-term virological suppression (6, 17). The clinical utility of measuring concentrations of NRTIs in plasma has not been established yet. NRTIs require intracellular phosphorylation to exert their antiretroviral activity. The measurement of intracellular triphosphate anabolite concentrations is technically complicated and currently possible only in research laboratories. The relationship between extracellular drug concentrations and intracellular phosphorylation is complex, and the correlation between concentrations in serum and intracellular triphosphate concentrations is weak or absent (10, 14). Nevertheless, availability of extracellular drugs is a prerequisite for subsequent phosphorylation of NRTIs.

Data show that plasma zidovudine (ZDV) and lamivudine (3TC) concentrations are stable between weeks 2 and 28 of therapy (7). As data on RTI concentrations in blood and cerebrospinal fluid (CSF) during prolonged antiretroviral therapy are not available, we evaluated whether the concentrations of the NRTIs ZDV, stavudine (d4T), 3TC, and abacavir (ABC) and the non-NRTI NVP in serum and CSF showed clinically relevant changes during 2 years of stable therapy.

Fifteen HIV-1-infected men, aged 31 to 60 years, were treated with ZDV (300 mg twice daily [BID]) (or d4T [40 mg BID]), 3TC (150 mg BID), ABC (300 mg BID), NVP (200 mg BID, after a 2-week lead-in of 200 mg once daily), and indinavir (IDV) (1000 mg three times daily) (16, 18). All but one patient were therapy naive at the start of treatment. In two patients ABC and NVP were stopped because of hypersensitivity reactions. In five patients ZDV was changed to d4T during the course of therapy because of toxicity. In four patients the NVP dosage was temporarily changed from 200 mg BID to 400 mg once daily. Since IDV dosages were adjusted in case of subtherapeutic drug concentrations, as previously described (16), IDV was not considered stable therapy and therefore was not included in the present study. Virological data have been described elsewhere (18).

A serum pharmacokinetic profile was obtained at weeks 8, 24, 48, 72, and 96. Blood was collected prior to drug administration (0 h) and at 1, 2, 4, 6, 8, and 10 h thereafter. Serum was obtained by centrifugation at 4°C for 20 min at 1,600 × g. A CSF sample was collected 1 h after administration, at weeks 8, 24, 48, and 96. All samples were stored at −70°C until analysis. The study was approved by the Medical Ethics Committee of our hospital, and informed consent was obtained from all patients.
Concentrations of ZDV, d4T, 3TC, ABC, and NVP in serum and CSF were measured by high-performance liquid chromatography or radioimmunoassay (ZDV and d4T) (11, 19, 20). The lower limits of quantification for ZDV, d4T, 3TC, ABC, and NVP in serum were 2.5, 20, 20, 18, and 25 ng/ml, respectively, and in CSF were 2.5, 20, 20, 5, and 5 ng/ml, respectively. The intraday and interday precisions of all assays were less than 15%.

The serum concentration (C) versus-time (t) data were analyzed by noncompartmental methods (3). The highest observed concentration was defined as peak concentration (Cmax), and the 12- and 24-h values for C (C12 and C24) were defined as trough concentrations (Cmin) for the BID and once-daily dosing, respectively. The terminal, log-linear period (log C versus t) was defined by the last data points (n/3) by visual inspection. The absolute value of the slope (β) was calculated by least-squares linear regression analysis. The concentrations at 12 and 24 h after ingestion of the drugs were calculated with the equations:

\[ C_{12} = C_{10} \cdot e^{-\beta t_{12}} \]
\[ C_{24} = C_{10} \cdot e^{-\beta t_{24}} - t_{12} \]

in the BID and once-daily regimens, respectively. The areas under the serum concentration-versus-time curve from 0 to 12 h (AUC0–12) and 0 to 24 h (AUC0–24) in the BID and once-daily regimens, respectively, were calculated by applying the trapezoidal rule from 0 to 12 h and 0 to 24 h, respectively. The NVP AUC0–24 in the BID regimen was calculated by multiplying the AUC0–12 by 2.

Pharmacokinetic parameters from weeks 8 to 96 were loge transformed to approximate normal distribution.

For the four patients who temporarily used NVP 400 mg once daily, only the AUC0–24 was included in the analyses, as the daily exposure to NVP as measured by the AUC0–24 is not different in a 400-mg-once-daily and a 200-mg-BID dosing regimen (15).

To assess changes over time and within- and between-patient variability in the Cmin, Cmax, AUC0–12, AUC0–24 and concentrations in CSF of the five drugs, random linear growth modeling was applied. To assess the most appropriate way to describe these data, two models within the random linear growth modeling procedure were used. The first was the intercept and slope model, in which intercept and slope were included as random effects (random slope model). This resulted in estimates of individual intercepts and slopes. The second model estimated only each patient’s intercept (random intercept model), assuming that all patients have identical slopes. The fit of the second model was compared to the first with a log-likelihood ratio test. If the random slope model did not have a significantly (P ≤ 0.05) better fit, the simpler random intercept model was used.

The calculated slope represents the change in loge-transformed pharmacokinetic parameters over time. The intercept is the intersection of each patient’s slope and the y axis. The within-patient variability in both models is the deviation of the pharmacokinetic parameters obtained at different time points from each patient’s individual slope. The between-patient variability in the random intercept model is expressed by the variability in intercepts. In the random slope model, the between-patient variability is given not only by the variability in intercept but also by the variability in slope. Data were analyzed with the SAS software package (version 8.0; SAS Institute, Cary, N.C.).

For all but five pharmacokinetic parameters, the random intercept model was found to be adequate. For the Cmax of 3TC and the concentration of NVP in CSF, the random slope model showed a significantly better fit. For three parameters (AZT Cmin and Cmax and NVP Cmin), the random linear growth model could not be fitted due to small sample size and/or large variability.

Nearly all parameters were relatively stable during 2 years of...
therapy (Fig. 1). The change over time varied from $-0.97$ to 0.57% per week, with only the ABC AUC$_{0-12}$ decreasing statistically significantly over time ($-0.41\%$ per week; 95% confidence interval, $-0.75$ to $-0.07\%$ per week) (Table 1). The within-patient and between-patient variabilities of $C_{\text{min}}$, $C_{\text{max}}$, AUC$_{0-12}$, AUC$_{0-24}$, and CSF are described in Table 1. In the case of a between-patient variability of 0%, all variability can be explained by the within-patient variability.

In conclusion, this study demonstrated that for most of the pharmacokinetic parameters, the within-patient variability was higher than the between-patient variability. Furthermore, the pharmacokinetic parameters of ZDV, d4T, 3TC, ABC, and NVP in blood and CSF do not change during prolonged therapy, suggesting that altered bioavailability and/or clearance of these drugs does not explain decreased virological efficacy during prolonged therapy. However, this does not preclude the possibility of less exposure to nucleoside analogues due to decreasing intracellular phosphorylation during prolonged antiretroviral therapy (1, 13).

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