Poster # 642

Frequent Sampling in Virologically Suppressed Patients Taking HIV Protease Inhibitors or Nonnucleoside Reverse Transcriptase Inhibitors Defines Intraindividual Pharmacokinetic Variability

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Abstract

Background: Effective therapeutic drug monitoring (TDM) for antiretrovirals (ARVs) requires a better understanding of intraindividual variability in pharmacokinetics (PK). Frequent repeated sampling of drug concentrations in an individual is usually not possible.

Methods: We were able to determine protease inhibitor (PI) and nonnucleoside reverse transcriptase inhibitor (NNRTI) concentrations in 10 patients with undetectable plasma HIV RNA (<50 c/mL) who were stable on their current regimen for at least 3 months. Subjects had plasma collected at the same time of day 3 times every week for up to 4 months in order to define the frequency of virologic "blips" (isolated plasma HIV RNA ≥50 c/mL). Patients were instructed to take their ARVs at the same time every day. Plasma samples for HIV RNA and drug concentration analysis were obtained simultaneously. Plasma PI and NNRTI concentrations were determined using validated HPLC methods. PK variance was expressed as intraindividual % coefficient of variation (ICV) = S.D./mean.

Results: Of 713 samples, blips occurred in 26 (3.6%) but did not coincide with low drug concentrations. ICV% was determined for a total of 17 drugs using 600 samples. ICV% was unexpectedly high in most patients taking PIs (lopinavir/ritonavir 24%, 33%, 52%, 85%; nelfinavir/M8 metabolite 30/44%, 38/52%; ritonavir 34%, 43%; saquinavir 52%, 55%). ICV% for NNRTIs was lower (efavirenz 7%, 13%, 28%, 51%; nevirapine 26%). Median ICV% for all PIs was 43% (n=12) and for all NNRTIs was 26% (n=5). Quality controls excluded laboratory artifact as a likely contributor to variance.

Conclusions: Using validated HPLC methods, intraindividual variance in the concentration of ARVs was surprisingly high in virologically suppressed patients. Possible contributors include food effects, concomitant use of prescription and herbal medications, or medication timing, which was assessed by self-report. High intraindividual PK variability may limit the utility of TDM for some ARVs in some settings.

Background

We used frequent repeated sampling of ARV drug concentrations to measure intraindividual variability in pharmacokinetics.

Patient Visit Timeline

Legend: 99, 113, 134, 135, 136, 140, 139, 147, 148, 154

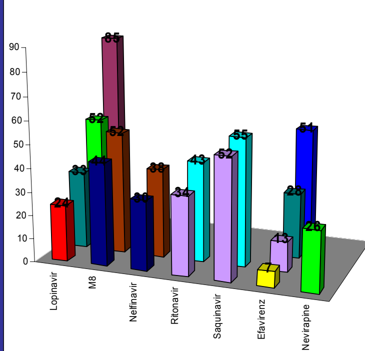
Days: 0, 20, 40, 60, 80, 100, 120

Methods

We determined PI and NNRTI concentrations in 10 patients with undetectable plasma HIV RNA (<50 c/mL) who were stable on their current regimen for at least 3 months. Subjects had plasma collected at the same time of day 3 times every week for up to 4 months. Patients were instructed to take their ARVs at the same time every day.

Plasma concentrations of lopinavir (LPV), ritonavir (RTV) and saquinavir (SQV) were determined using a modified validated high-performance liquid chromatography (HPLC) assay[1]. The internal standard (IS), A-86093.0, was supplied by Abbott Laboratories (Abbott Park, IL) and the mobile phase was 0.1% trifluoroacetic acid, acetonitrile and methanol (53:42:5). Analytes were separated isocratically followed by a step gradient wash at 30°C using a reverse-phase Beckman C18 column, and detected at 220 nm (IS and LPV) and at 239 nm (IS, RTV and SQV). Calibration standards ranged from 100 to 15,000 ng/mL for LPV and RTV and 87 to 13,121 ng/mL for SQV. Plasma concentrations of nelfinavir (NFV), the NFV active metabolite M8, and efavirenz (EFV) were determined using a validated HPLC assay. Plasma proteins were precipitated with acetonitrile and the supernatant was dried. Samples were dissolved in mobile phase, applied to a C18 reverse-phase column (Beckman) at 30°C, separated isocratically in 0.1% trifluoroacetic acid, pH 5.0:acetonitrile:methanol (47:48:5), and detected at 253 nm. Calibration standards ranged from 100 to 20,000 ng/mL. Plasma concentrations of nevirapine (NVP) were determined using a modified validated HPLC assay[2]. Plasma samples were applied to a Waters HLB reverse-phase cartridge, washed with an acid/base series, eluted dried, and dissolved in mobile phase (63% 25 mM phosphate buffer (pH 6.0) and 5.2 mM 1-butanesulfonic acid, 21.5% methanol and 15.5% acetonitrile). Analytes were separated isocratically followed by a step gradient wash at 30°C using a reverse-phase Supelco™ LC-8 column, and detected at 280 nm. Calibration standards ranged from 25 to 10,000 ng/mL.

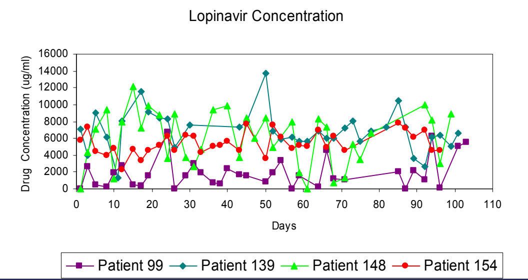
For all assays, quality control samples were interspersed between unknown samples. Mean correlation coefficients for calibration curves were ≥0.998 ± 0.001. The precision and accuracy for all assays were high, with coefficients of variation (C.V.) of < 13 % intra-day and < 8 % inter-day. PK variance was expressed as intraindividual % coefficient of variation (ICV) = standard deviation/mean x 100.

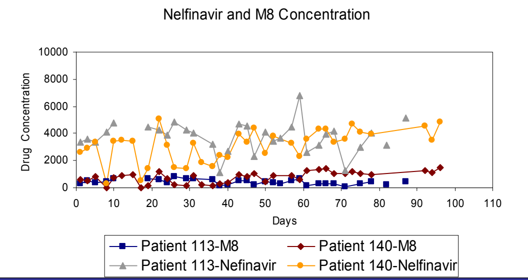


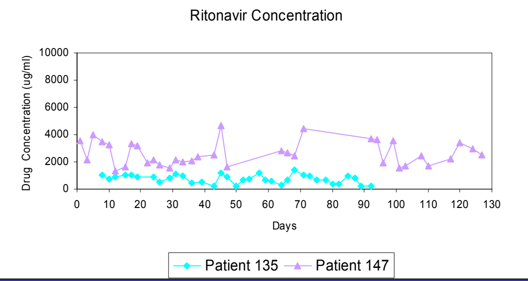
Results

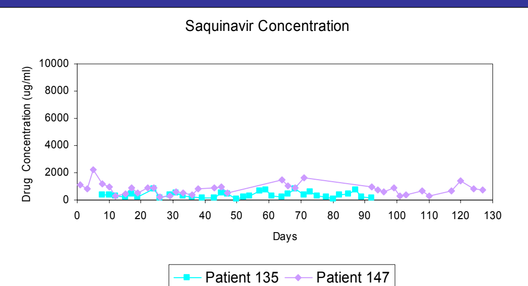
Of 713 samples, blips occurred in 26 (3.6%) but did not coincide with low drug concentrations[3]. ICV% was determined for a total of 17 drugs using 600 samples. ICV% was unexpectedly high in most patients taking PIs (chart to left). ICV% for NNRTIs was lower (chart to left). Median ICV% for all PIs was 43% (n=12) and for all NNRTIs was 26% (n=5). Quality controls excluded laboratory artifact as a likely contributor to variance.

Protease Inhibitor Concentrations over Time

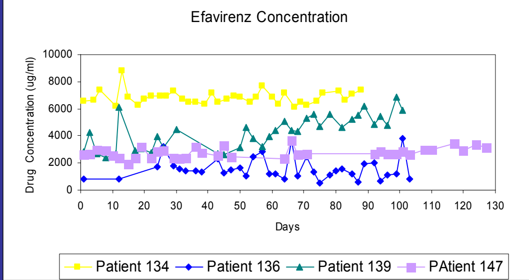


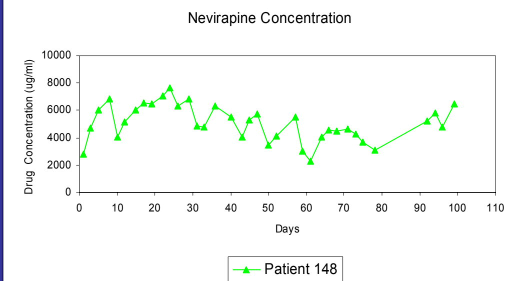






Nonnucleoside Reverse Transcriptase Inhibitor Concentrations over Time





Discussion

Frequent sampling of PI and NNRTI drug concentrations in this cohort of virologically suppressed patients reveals surprising high intraindividual variance in ARV concentrations. This suggests that providers should use caution when making clinical decisions based on limited ARV concentration measurements. This finding must be tempered in that the study was a prospective observational study with adherence monitored by self-report only. The dose timing is critical to interpretation of these data and was also by self-report. Non-adherence could have contributed to some of the variance in drug concentrations, but was not sufficient to result in sustained virologic rebound. The fact that most of these subjects reported to clinic at the same time every MWF for 3-4 consecutive months is evidence for motivation and effort applied to keep clinic appointments, and by association suggests a highly adherent group of subjects. Laboratory artifact is a possible contributor to variance. Quality control and quality assurance techniques applied to all assays in this study diminish but do not eliminate the possibility of laboratory artifact. We would anticipate that any analytical laboratory employing similar QA/QC would be susceptible to the same artifact.

Conclusions

1. Intraindividual variance in the concentration of ARVs was surprisingly high in virologically suppressed patients.
2. Possible contributors include food effects, concomitant use of prescription and herbal medications, or medication timing, which was assessed by self-report. All of these factors will need to be considered by clinicians making treatment decisions based on TDM without frequent sampling.
3. High intraindividual PK variability may limit the utility of TDM for some ARVs in some settings.

References

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2. Pav JW, Rowland LS, Korpalski DJ. HPLC-UV method for the quantitation of nevirapine in biological matrices following solid phase extraction. J Pharm Biomed Anal 1999; 20(1-2): 91-98.
3. Nettles RE, Kieffer TL, Kwon P, et al. "Intermittent HIV-1 Viremia (Blips) and Drug Resistance in Patients Receiving HAART." JAMA. 2005;293:817-829.