NOTES

Immunochromatographic Strip Test for Rapid Detection of Nevirapine in Plasma Samples from Human Immunodeficiency Virus-Infected Patients[∇]

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We report a novel one-step immunochromatographic strip test for the rapid, qualitative detection of nevirapine in plasma samples from human immunodeficiency virus-infected patients. The sensitivity was 100% (95% confidence interval [95% CI], 97.8 to 100%), and the specificity was 99.5% (95% CI, 97.2 to 99.9%). The limit of detection was 25 ng/ml. Immunochromatographic strip tests are simple, rapid, and cheap assays that could greatly facilitate drug level monitoring in resource-limited settings.

To date, insufficient data exist to recommend the routine monitoring of levels of antiretroviral drugs in plasma to optimize therapy, but there are certain situations, such as drug-drug interactions, changes in physiology, pregnancy, drug adherence, and the management of drug toxicities, in which the measurement of antiretroviral drug levels may be beneficial for patient management (10). However, even in resource-rich settings, antiretroviral drug level measurement has been restricted due to the limited availability of laboratories performing the quantitative “gold standard” high-performance liquid chromatography (HPLC) assays. To facilitate access to drug level monitoring for patients in resource-limited settings, simpler and cheaper methods are needed. Immunochromatographic (IC) strip tests are simple, rapid, and cheap assays which have been developed for the qualitative measurement of many biological markers. Recently, we developed a one-step IC strip test for the detection of nevirapine (NVP) in plasma (9). In this study, we report the sensitivity and specificity of this IC strip test for the qualitative detection of NVP in plasma samples from human immunodeficiency virus (HIV)-infected patients.

Stored plasma samples were randomly selected for assessment by the IC strip test. Samples from two source groups were selected: (i) women who participated in the Program for HIV Prevention and Treatment clinical trial 2 (PHPT-2) (8) and were exposed to single-dose NVP (SD-NVP) for the prevention of mother-to-child transmission (PMTCT) of HIV and (ii) patients receiving highly active antiretroviral therapy (HAART), with and without (negative controls) NVP, whose

samples were sent to the Faculty of Associated Medical Sciences laboratory at Chiang Mai University, Chiang Mai, Thailand, for antiretroviral therapeutic drug monitoring (TDM). The antiretroviral drug levels in all plasma samples were quantified previously by a validated HPLC assay (4) at the Faculty of Associated Medical Sciences laboratory, which participates in the U.S. AIDS Clinical Trials Group Pharmacology Quality Control Program. The lower limit of NVP quantification by HPLC was 50 ng/ml.

The IC strip test we developed is a competitive assay (9). All reagents for the strips were prepared at the Faculty of Associated Medical Sciences laboratory, and they were assembled by i+MED Laboratories Co. Ltd. (International Standards Organization 13485:2003 certified), Thailand. Briefly, on a nitrocellulose membrane, NVP conjugated to bovine serum albumin was streaked at the test line, goat anti-rabbit immunoglobulin G was streaked at the control line, and dried rabbit anti-NVP polyclonal antibodies conjugated to colloidal gold particles (anti-NVP-CGC) were placed onto a conjugate pad. Dipping the strip into plasma dissolves the anti-NVP-CGC, and through capillary action, the plasma and anti-NVP-CGC migrate along the surface of the membrane. In the absence of NVP, anti-NVP-CGC bind to NVP-bovine serum albumin and goat anti-rabbit immunoglobulin G at the test and control lines, respectively, and red-purple bands of equal intensities appear at both lines due to the accumulation of colloidal gold (Fig. 1, first lane). In the presence of NVP, anti-NVP-CGC are neutralized by free NVP in the plasma, and the intensity of the color at the test line fades as the concentrations of NVP increase (Fig. 1, second through fifth lanes), but a band appears at the control line independently of the presence of NVP. At therapeutic NVP concentrations (>3,400 ng/ml), no band is visible at the test line. However, as the NVP concentration decreases, a weak band appears at the test line. The IC strip

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FIG. 1. IC strip test results for plasma samples collected from HIV-infected patients and containing different concentrations of NVP. Limit of detection, 25 ng/ml. The upper band is the control line, and the lower band is the test line.

limit of detection was set at 25 ng/ml in order to limit the subjectivity required to determine a decrease in the intensity at the test line compared to that at the control line. IC strip results were valid if a strong band developed at the control line within 5 min.

All plasma samples selected were identified by a unique identification code, and the laboratory technicians conducting the IC strip tests were blinded to the drugs present. All samples were assessed at room temperature (20 to 25°C). The sensitivity, specificity, and exact 95% binomial confidence intervals (95% CI) were calculated using Stata software (version 9.2; StataCorp, United States). This study was approved by the ethics committees at the Faculty of Associated Medical Sciences, Chiang Mai University.

A total of 204 plasma samples from patients who were exposed to NVP and 196 plasma samples from patients not exposed to NVP were selected. Of the 204 samples, 163 (80%) had NVP levels detectable by HPLC (median concentration, 2,916 ng/ml; range, 51 to 41,216 ng/ml). Compared to the HPLC method to detect NVP, the sensitivity of the IC strip was 100% (163 of 163; exact one-sided 97.5% CI, 97.8 to 100%) and the specificity was 99.5% (195 of 196; exact 95% CI, 97.2 to 99.9%).

Forty-one samples with NVP levels undetectable by HPLC were from patients exposed to NVP. Of these samples, 11 had NVP levels detectable by the IC strip test. In all likelihood, the lower limit of detection of the IC strip explains these results, as very low NVP concentrations in these samples were expected: 10 were postpartum samples collected 9 to 11 days after expo-

sure to SD-NVP, and 1 sample was taken 2 weeks after stopping of an NVP-containing HAART regimen. Samples with NVP levels undetectable by HPLC and the IC strip were mainly postpartum samples collected more than 10 days after exposure to SD-NVP.

Studies have reported a relationship between plasma NVP levels and efficacy in developed countries (11); however, pilot studies have failed to demonstrate that routine TDM of NVP is beneficial (3, 7). In resource-limited settings where NVP is a common component of first-line antiretroviral regimens, the effectiveness of TDM of NVP to help optimize antiretroviral therapy remains to be investigated. Our recently developed qualitative strip test, at an estimated cost of \$4 per strip, may facilitate drug level monitoring in these settings. For example, NVP drug level assessment using the IC strip may serve as an additional tool to help monitor drug adherence (6), as well as significant drug-drug interactions (1). Although, the IC strip described in this report is unable to provide information with respect to the minimum effective concentration of NVP, it can still provide useful drug adherence information, particularly if no drug is detected. Semiquantitative IC strip tests with the capacity to identify minimum effective drug concentrations (i.e., concentrations above or below the minimum effective concentration) would be ideal and are a priority for future development.

The application of the IC strip to qualitatively detect NVP may also be useful in the context of PMTCT programs. Presently, SD-NVP remains a key part of the recommended antiretroviral prophylaxis regimen for PMTCT in many settings (12), and the measurement of NVP levels in umbilical cord blood samples has been suggested as a method to assess the effectiveness of PMTCT programs (5). Indeed, a two-step testing algorithm incorporating a qualitative thin-layer chromatography method and HPLC to detect NVP in cord blood samples has been successfully tested as an approach to measure the effectiveness of SD-NVP PMTCT programs (2); however, a limitation of the thin-layer chromatography method is that the concomitant use of zidovudine confounds the results. Phase I studies have demonstrated that peak maternal and infant plasma NVP levels are between 1,500 and 2,000 ng/ml; therefore, the IC strip test we developed could easily be incorporated in these settings to help evaluate these large-scale PMTCT programs.

In the same context, based on current strategies of providing supplemental postpartum antiretroviral therapy following SD-NVP until NVP concentrations are no longer sufficient to try to prevent the selection of nonnucleoside reverse transcriptase inhibitor-resistant viruses, the IC strip could be used on a patient-by-patient basis to help determine when the postpartum antiretroviral therapy could be stopped. This rationale could also be applied to patients interrupting an NVP-containing HAART regimen.

In conclusion, the newly developed NVP IC strip test has excellent sensitivity and specificity compared to HPLC. Its major advantages over HPLC assays are its significantly lower cost (approximately \$4/test versus \$45/test) and its applicability in settings with limited laboratory infrastructure. As an alternative to HPLC methods, IC strip tests could facilitate TDM in resource-limited settings, and continued development of these

strip tests for the measurement of antiretroviral drugs is warranted.

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