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Can Urine Lamivudine Be Used to Monitor Antiretroviral Treatment Adherence?

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Abstract

Patient adherence to treatment is an important factor in the effectiveness of antiretroviral regimens. Adherence to treatment could be monitored by estimation of antiretroviral drugs in biological fluids. We aimed to obtain information on the quantity and duration of excretion of lamivudine in urine following oral administration of a single dose of 300 mg and to assess its suitability for adherence monitoring purposes. Spot urine samples were collected before dosing and at 4, 8, 12, 24, 28, 32, 48, 72, and 96 hours post dosing from 10 healthy subjects, and lamivudine was estimated by high-pressure liquid chromatography (HPLC). Lamivudine values were expressed as a ratio of urine creatinine. About 91% of the ingested drug was excreted by 24 hours, and the concentration thereafter in urine was very negligible. A lamivudine value of 0.035 mg/mg creatinine or less at 48 hours is suggestive of a missed dose in the last 24 hours. The study findings showed that estimation of urine lamivudine in spot specimens could be useful in monitoring patient adherence to antiretroviral treatment. However, this needs to be confirmed on a larger sample size and among patients on once-daily and twice-daily treatment regimens.

Introduction

Highly active antiretroviral therapy (HAART) allows patients who are infected with HIV to live productive and relatively disease-free lives for long periods. HAART is composed of various classes of antiretroviral drugs. The current standard care for the treatment of HIV-1 infection is a triple-drug therapy with 2 nucleotide or nucleoside reverse transcriptase inhibitors (NRTIs) forming the backbone in combination with a nonnucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor.[1,2]

Fixed-dose combinations (FDCs) of antiretroviral drugs are widely used as first-line regimens in India, Africa, and other developing/resource-constrained areas.[3,4] Two combinations, zidovudine/nevirapine/lamivudine (ZDV/NVP/3TC) and stavudine/nevirapine/lamivudine (d4T/NVP/3TC), are available as FDCs in the developing world. The advantages of using FDCs include convenience, reduction in prescription errors, reduced pill counts, and potential for improved adherence.

Variability in response to antiretroviral agents has been attributed in part to virologic, immunologic, pharmacokinetic factors and adherence differences between patients.[5] Adherence to antiretroviral treatment is a strong predictor of virologic suppression, disease progression, and death.[6-9] Clinical trials have suggested that early and late virologic failures appeared to be related

more to adherence issues and the potency of the regimen rather than the emergence of drug-resistant viruses.[10,11] Hence, monitoring patient adherence to treatment is important to ensure optimal outcomes. Identifying accurate predictors of adherence that can routinely be applied in clinical practice will be of value.

Currently available approaches to measure adherence include (1) patients' self-report, (2) physician assessments, (3) electronic monitoring, (4) pill count, and (5) prescription-refill compliance. Although these methods have proved to be predictive of outcomes, the results are variable.[7] Some investigators have assessed antiretroviral drug levels in the blood as a measure of adherence.[12,13] Alternatively, urine could serve as a useful biological fluid for measuring antiretroviral drug levels, particularly to monitor patient adherence to treatment, if found feasible. Such a method would be noninvasive and simple to perform. This method is applied in tuberculosis therapy, in which the detection of acetyl isoniazid in urine indicates intake of isoniazid within the past 24 hours.[14]

In a study carried out at our center, it was observed that a single oral dose of NVP administered to healthy subjects was excreted in urine for up to 9 days. This could be due to the long half-life of NVP (3035 hours). It was therefore apparent that urine NVP would not be a useful predictor of antiretroviral adherence.[15] We hypothesized that a similar approach could be tested with other antiretroviral drugs, which have a shorter half-life and could be detected easily in urine.

3TC (2'-deoxy-3'-thiacytidine), a cytosine nucleoside analog, is being effectively used in combination with other antiretroviral drugs to treat HIV-1 infection. It is potent against HIV, well tolerated, and does not require any rigorous schedule with respect to food. 3TC could serve as a useful candidate for monitoring patient adherence to antiretroviral treatment because it has several advantages: It is present in all FDC pills; it has a shorter elimination half-life than NVP (about 57 hours); the primary route of elimination is renal; and the major portion (70%) of an oral dose is excreted unchanged as a parent compound.

3TC is a prodrug and undergoes phosphorylation by intracellular kinases to form 3TC-5'-triphosphate, the active metabolite that prevents viral replication. This compound has a long intracellular half-life of 15.5 hours. Because 3TC requires intracellular activation, it has been hypothesized and proved that the intracellular level of the active triphosphate metabolite rather than unchanged drug levels in plasma correlate with virologic response in HIV-infected patients.[16] It has also been shown that 3TC, when administered at the recommended dosage of 150 mg twice daily, produces serum concentrations consistently above the in vitro IC_{50} against HIV-1 in various cell lines.[17]

In order to assess the feasibility of using 3TC detection in urine as a predictor of antiretroviral treatment adherence, it is important to obtain information on the amount and extent of excretion of a single dose of the drug. Very limited information is available on the pattern of urinary excretion of 3TC, and no attempts have been made to use this as a test of adherence. This, however, requires a simple method to estimate 3TC in urine. Morris and Selinger[18] have described a high-pressure liquid chromatographic (HPLC) method for determination of 3TC in urine, which allows direct injection of urine with column switching. This method requires 2 columns, one for getting rid of unwanted urine constituents and the other for elution of 3TC and analysis. We have developed a simple method for estimation of 3TC concentrations in urine by direct injection of suitably diluted urine (1:10/1:50) and analysis with a 150-mm column with UV detection. This method was applied to estimate urine 3TC concentrations in the urine of healthy volunteers who were administered a single oral dose of 300 mg of 3TC. The aim of the study was to obtain information on the quantity and duration of excretion of 3TC in urine and assess its suitability for adherence monitoring purposes.

Methods

Estimation of Urine 3TC by HPLC

Chemicals

3TC tablets (*Retrolam* 150) were obtained from Alkem Laboratories Ltd., India. Pure 3TC powder was a kind gift from Aurobindo Pharma, Hyderabad, India. Methanol (HPLC-grade) and potassium dihydrogen orthophosphate were purchased from Qualigens, India. Deionized water was processed through a Milli-Q water purification system (Millipore, Billerica, Massachusetts).

Chromatographic System

The HPLC system (Shimadzu Corporation, Kyoto, Japan) consisted of 2 pumps (LC-10ATvp), diode array detector (SPD-M10Avp), and system controller (SCL-10Avp). A rheodyne manual injector (Rheodyne, Cotati, California) attached with a 20-microliter (mcL) sample loop was used for loading the sample. ClassVP-LC workstation was used for data collection and acquisition. The analytic column was a C_{18}, 150 × 4.6 mm inner diameter, 5-micron particle size (Lichrospher 100 RP-18e, Merck, Germany) protected by a compatible guard column.

The mobile phase consisted of 50 mM phosphate buffer pH 4.0 and methanol (85:15 volume/volume) containing 0.05% triethylamine. Prior to preparation of the mobile phase, the phosphate buffer and methanol were degassed separately with a Millipore vacuum pump. The UV detec-

tor was set at 254 nm. The chromatogram was run for 6 minutes at a flow rate of 0.75 mL/minute at ambient temperature. Unknown concentrations were derived from linear regression analysis of the peak height vs concentration curve.

3TC Concentration Curve

The concentration curve was set up with a set of 3TC standards ranging from 2.5 to 50.0 micrograms (mcg)/mL. The standards were prepared from a stock solution with suitably diluted normal 3TC-free urine. The linearity of the standard concentrations was verified with estimates of the correlation coefficient (*r*). The intraday and interday variations of 3TC standards were determined by processing each standard concentration in duplicate for 6 consecutive days.

Assay Specificity

Interference from endogenous compounds was investigated by analyzing blank urine samples obtained from 6 male and 6 female subjects. Interference from certain antiretroviral drugs, namely, NVP, efavirenz, ZDV, didanosine, d4T, indinavir, and nelfinavir; antituberculosis drugs, such as rifampicin, isoniazid, pyrazinamide, ethambutol, streptomycin; and other commonly coadministered medications, such as ofloxacin, acetozolamide, loperamide, prednisolone, diphenylhydantoin, amitriptyline, cotrimoxazole, and fluconazole at a high concentration of 50 mcg/mL, was also evaluated.

3TC Stability in Urine

The stability of 3TC in human urine when stored at -20°C was evaluated by assaying 10 urine samples containing 3TC on days 1 and 7.

Study in Healthy Volunteers

Ten adult, healthy volunteers aged 18 years and older, including 7 men and 3 women, took part in the study. Their mean age and body weight were 39 years and 63 kg, respectively. All of the volunteers underwent physical examination by a medical officer. None of the volunteers had been suffering from any illness or taking concurrent medications at the time of the study. The purpose of the study was explained to the study participants, and only those who were willing to participate were included. Informed written consent was obtained from all of the study participants before they took part in the study. Smokers, chronic alcoholics, and women on hormonal birth control pills were not included in the study.

All of the volunteers were requested to report to the clinic division of the Tuberculosis Research Centre, Chennai, India, in the morning after an overnight fast. On the day of the study, a sample of urine was collected (0 hour) in a labeled container. Two tablets of 150 mg 3TC (300 mg) were administered in about 200 mL of water. They were instructed to collect spot urine samples at 4, 8, 12, 24, 28, 32, 48, 72, and 96 hours after drug administration. All of the urine samples were stored at -20°C until assay. The 3TC concentration in the urine samples was estimated according to the method described in this study. 3TC concentrations were expressed as a ratio of urine creatinine concentration. Urine creatinine was estimated by a colorimetric method that is based on Jaffe's reaction.[19]

Results

The calibration curve parameters of 3TC from 6 individual experiments for standard concentrations ranging from 2.5 to 50.0 mcg/mL showed a linear relationship between peak height and concentration. The correlation coefficient (*r*) values ranged from 0.99577 to 0.99999. The linearity and reproducibility of the various standards used for constructing calibration graphs for urine 3TC are given in Table 1 . The intraday and interday relative standard deviation (RSD) for standards 2.550.0 mcg/mL ranged from 0.3% to 4.0% and 3.2% to 7.2%, respectively. The accuracy of the method was 102%. The mean urine 3TC concentrations measured on days 1 and 7 in 10 urine samples

Table 1: Linearity and Reproducibility of Urine Lamivudine Standards

	Mean Peak Height ± SD (RSD %)	
Standard Concentration (mcg/mL)	**Intraday (n = 6)**	**Interday (n = 6)**
2.5	7948 ± 211 (2.7)	8019 ± 571 (7.1)
5.0	15,534 ± 308 (2.0)	15,424 ± 1110 (7.2)
12.5	40,042 ± 1388 (3.4)	37,916 ± 2743 (7.2)
25.0	75,985 ± 3063 (4.0)	72,825 ± 4733 (6.5)
50.0	152,533 ± 388 (0.3)	151,735 ± 4824 (3.2)

mcg = micrograms; RSD = relative standard deviation; SD = standard deviation

stored at -20°C were 106.6 and 101.6 mcg/mL. No degradation of 3TC in urine occurred up to 1 week when stored at -20°C.

Urine 3TC concentrations were calculated from the calibration standard curve and multiplied by the appropriate dilution factor. In order to account for variations in spot urine volume, all 3TC values were expressed as a ratio of creatinine concentration. Table 2 gives the 3TC values per milligram of creatinine (mean and range) obtained in 10 healthy subjects.

Discussion

In most cases, HAART results in a reduction in plasma viral load to below the limit of detection. Regardless of the decrease in morbidity and mortality associated with HAART regimens and the significant increase in the life expectancy of treated HIV-infected individuals, eventual failure of therapy is common and poses challenges for future treatment. The failure of HAART most likely arises from a combination of viral and host factors that facilitate the emergence of HIV variants with resistance to multiple antiretroviral drugs. The emergence of drug resistance in patients receiving HAART can be primarily attributed to the high spontaneous mutation rate and high rate of HIV turnover in HIV-infected individuals, selective pressure arising from antiretroviral therapy, pharmacokinetic characteristics of antiretroviral drugs, patient tolerance/adherence to antiretroviral regimens, and the existence of viral reservoirs.[20]

Patient adherence is a highly important factor in the effectiveness of antiretroviral regimens, and affects the evolution of viral variants with different degrees of sensitivity to drugs.[21] Theoretically, total adherence should prevent the emergence of resistant strains, but incomplete patient adherence coupled with an array of other pharmacologic factors results in the presence of a heterogeneous population, and the possibility of selecting for viral resistance. Many factors influence the degree of patient adherence to therapy, such as side effects of drugs (toxicity), high costs of antiretroviral regimens, and lack of infrastructure needed to monitor their use. Currently available approaches to measure adherence have notable limitations,[22] and individual patient assessments by medical providers do not accurately predict adherence.[23] Liechty and coworkers[24] reported that an abnormally low, untimed antiretroviral drug level can identify individuals with very low adherence at high risk for HIV disease progression and death.

Monitoring compliance by measuring the presence of indinavir in saliva has been reported.[25] A similar approach with respect to urine levels of antiretroviral drugs would be useful in monitoring adherence. Urine collections are noninvasive and would be most suited to the patients. In this study, we attempted to assess whether a simple spot urine test for 3TC could help in monitoring patient adherence to antiretroviral treatment. The reason for choosing 3TC was that, apart from having a short elimination half-life, it is present in the fixed-dose triple-/double-drug combination of antiretroviral drugs commonly used in resource-limited settings.

Information on analytic methods for estimation of urine 3TC is very limited. The method of Morris and Selinger[18] allows direct injection of urine with HPLC column switching followed by UV detection. They performed

Table 2: Lamivudine Concentrations in Spot Urine of 10 Healthy Subjects

Time After Drug Administration (hours)	Mean (Range) (mg Lamivudine/mg Creatinine)
4	0.68 (0.270.99)
8	0.31 (0.150.46)
12	0.088 (0.0560.12)
24	0.036 (0.0120.059)
28	0.034 (0.0090.046)
32	0.024 (0.0060.035)
48	0.019 (0.0030.027)
72	0.015 (00.022)
96	0.009 (00.016)

online extraction with a Spherisorb SCX column (Waters Corporation, Milford, Massachusetts) that was eluted with deionized water. 3TC was retained in the column, whereas the bulk of the urine constituents was eluted to waste. The SCX column was then backflushed to a BDS-Hypersil-C18 column (Keystone Scientific, Bellefonte, Pennsylvania) and eluted with a mobile phase consisting of acetate buffer and methanol. Direct injection of urine to the analytic column would damage it and shorten its life. One way to overcome this problem is by column switching, as reported by Morris and Selinger.[18] However, this could be time-consuming and cumbersome. Alternatively, urine could be adequately diluted and then directly injected into the analytic column. This makes the method very simple and rapid without causing any damage to the column.

We made an attempt to standardize urine 3TC estimation by suitably diluting the urine and directly injecting the diluted urine into the HPLC column. Under the chromatographic conditions described above, 3TC was resolved as a single discrete peak at 3.5 minutes.

Because HIV-infected individuals receive treatment for various opportunistic infections, it is important to establish the specificity of the method. No endogenous compounds or antiretroviral drugs, such as NVP, efavirenz, ZDV, didanosine, dT4, indinavir, and nelfinavir; antituberculosis drugs, such as rifampicin, isoniazid, pyrazinamide, ethambutol, streptomycin; and other commonly coadministered medications, such as ofloxacin, acetozolamide, loperamide, prednisolone, diphenylhydantoin, amitriptyline, cotrimoxazole, and fluconazole, interfered in the 3TC chromatogram (data not shown).

The method was applied for the determination of 3TC concentration in spot urine collected at different time points from 10 healthy subjects after they were administered a single oral dose of 300 mg 3TC. The reason for selecting the 300-mg once-daily dose was that many regimens are now designed for once-daily use in order to improve patient adherence. As expected, there was a steady decline in 3TC concentrations excreted in urine with time (see Table 2). A major portion of the drug is excreted by 8 hours, and very negligible amounts of 3TC up to 96 hours. In order to monitor patient adherence on a once-daily regimen, an ideal test should be positive up to 24 hours and negative beyond this period. In the case of twice-daily regimens, the test should be negative after 12 hours. The mean 3TC values at 24 and 28 hours were 0.036 and 0.034 mg/mg creatinine, respectively. While keeping a cutoff value of 0.035 mg (mean of above 2 values), it was observed that 2 patients each at 24 and 28 hours and 1 patient at 32 hours had 3TC concentrations exceeding the cutoff value, and none at 48 hours and beyond. Therefore, a 3TC concentration of 0.035 mg/mg creatinine or less at 48 hours is suggestive of a missed dose the previous day.

The study, which was conducted on a small number of healthy subjects, has provided information on the extent of excretion of a single dose of 300 mg 3TC. About 91% of the ingested drug is excreted by 24 hours. The concentration thereafter is very low. Patients undergoing antiretroviral treatment would be at steady state and excreting slightly higher concentrations of the drug. The findings of this study showed that estimation of 3TC in spot urine could be useful in monitoring patient adherence to antiretroviral treatment. However, these findings need to be confirmed on a larger sample size among patients on once-daily and twice-daily treatment. A simple urine test would go a long way in monitoring antiretroviral adherence in resource-constrained settings.

Authors and Disclosures

Agibothu K. Hemanth Kumar, PhD, has disclosed no relevant financial relationships.

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