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Therapeutic Drug Monitoring for Antiretroviral Therapy: Usefulness and Limitations

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Abstract

The role of therapeutic drug monitoring (TDM) in the management of antiretroviral drugs is a topic of increasing interest. If TDM is to be incorporated into patient care, it is essential that randomized controlled trials demonstrate that this intervention is both of clinical benefit for the patient and cost-effective. Such trials are in progress. Even without these data it makes intuitive sense to at least ensure drug concentration is maintained above certain levels to get optimal antiviral effect. Since nucleoside reverse transcriptase inhibitors (NRTIs) have to be intracellularly phosphorilated and the correlation between plasma nucleosides and intracellular triphosphates is poor, most attention has focused on the protease inhibitors (PIs). Arguments in favour of TDM for PIs include: Low drug exposure correlates with poor virological response, marked inter-individual variability in drug concentrations, complex drug interactions, and high plasma levels correlating with toxicity. However, it is unclear which PK values (Cmax, Cmin, AUC) are the most important to determine, although preliminary data support the use of single trough levels as a good parameter for this purpose. For non-nucleoside reverse transcriptase inhibitors (NNRTIs), blood drug levels are almost always more than 10- to 100-fold above the IC_{50} when used at the recommended doses. However, recent clinical trials have underlined that critical blood levels, which are 1-2 logs above the *in vitro* IC_{50}, need to be reached if long-term viral suppression is to be achieved. If this is the case, TDM might also be useful when using NNRTIs.

Key words

Therapeutic Drug Monitoring. Antiretroviral therapy. Drug levels.

Introduction

Recent advances in the treatment of HIV-1 infection involving the coadministration of reverse transcriptase and protease inhibitors has permitted the achievement of nearly complete suppression of HIV replication. As a consequence, considerable improvements in the life expectancy of infected patients have been noticed[1]. Highly active antiretroviral therapy (HAART) has become the standard of care[2]. Plasma HIV-RNA (viral load) and the CD4+ T-lymphocyte counts are the most important parameters for guiding when to start treatment and for monitoring the response to therapy. Both are independent markers of therapeutic success[3].

Incomplete viral suppression in the context of insufficient drug pressure inevitably leads to the

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appearance of HIV variants resistant to drugs. Non-adherence to antiretroviral therapy is particularly critical because it allows continued viral replication and the development of drug resistance[4,5]. Mutations at specific sites in the reverse transcriptase or in the protease genes have been associated with reduced sensitivity to antiretroviral drugs. Moreover, a good correlation has been found between resistance mutations and a decreased sensitivity in phenotypic assays.

Several factors lead to nonadherence to antiretroviral therapy: Large numbers of pills to be taken per day, the development of toxicities, and the varying dosage regimens are among the most relevant[6]. But not all virological failures can be attributed to the development of resistant viruses. Drug related factors, which can explain failure, include malabsorption, insufficient dosage, drug-drug interactions, impaired intracellular metabolism, hyperactivity of the cytochrome P450, overexpression of the P- glycoprotein, and the interpatient variability in metabolism and clearance[7]. These factors can result in suboptimal drug concentrations with subsequent incomplete viral suppression, and the development of resistance. On the other hand increased blood concentrations may result in toxicity.

Therapeutic drug monitoring (TDM) has been proposed as a useful tool for the optimisation of antiretroviral therapy, allowing an effective drug concentration for each treated individual. In this review, we will discuss the current role of TDM in clinical practice as well as its possible limitations.

Evidences favouring the use of TDM

Not all three classes of antiretroviral drugs currently available for the treatment of HIV infection have the same possibilities to be monitored. Their different pharmacokinetics and pharmacology lead them to be considered in a different way[8].

Nucleoside Retrotranscriptase Inhibitors

Nucleoside retrotranscriptase inhibitors (NRTIs) require an intracellular activation step[9], which consists in a triple phosphorilation carried out by patient's kinases. Several studies have demonstrated the importance of host cellular factors as a cause of treatment failure. Particularly, the importance of thymidin kinase activity has been associated to a decrease in NRTI susceptibility in patients exposed to these drugs for long periods of time[10-13]. A correlation between intracellular phosphorilated drug concentrations and response to therapy has been demonstrated in several studies. In 19 patients treated with d4T and 3TC, intracellular concentrations of 3TC-TP and d4T-TP were measured in peripheral blood mononuclear cells. Patients were included in three groups: group 1, NRTI naive and responders (R); group 2, long-term ZDV experienced patients and non-responders (Non-R), and group 3, long-term ddI pre-treated and short-term or non-compliant to ZDV treatment. NRTI phosphorylation correlated significantly with response in R and Non-R in each group. Following long-term ZDV treatment, Non-R was associated with a significant decrease in d4T and 3TC phosphorilation[14]. Thus, intracellular levels of the phosphorilated forms of d4T, AZT and 3TC were correlated with an improved outcome[15]. No correlation was found between phosphorilated intracellular concentrations and plasma levels of these drugs.

The benefit of maintaining a concentration-controlled therapy has been demonstrated in several trials. In a 24-week double-blinded, crossover study, AZT (100 mg 5 times daily) was compared with "concentration-controlled" AZT therapy, defined as the dose necessary to maintain a plasma AZT concentration of 0.18 μg/mL. Overall, the concentration-controlled regimen achieved higher systemic concentrations with reduced interpatient variability. The steady-state average plasma concentrations of ZDV were 0.2 μg/mL versus 0.16 μg/mL for the standard regimen. There was no difference in safety and tolerance between the regimens. Intracellular ZDV-triphosphate concentrations averaged 160 fmol/106 PBMC in concentration-controlled patients versus 92 fmol/106 PBMC in those under standard therapy. An increase of 22% in the CD4+ cell count was noticed in the concentration-controlled arm versus a 7% decrease in those under the standard management. These data indicate that pharmacologic variability clearly affects the antiretroviral response[16].

In another study using concentration-controlled oral ZDV therapy, similar results were obtained, indicating that concentration controlled oral antiretroviral therapy with ZDV is feasible and safe, and provides pharmacologic data to predict the virologic and inmunologic benefits[17].

In conclusion, TDM of NRTIs can help to increase the efficacy of antiretroviral combination therapy including NRTIs. However, more rapid, precise, sensitive and cheaper methods for quantifying intracellular compounds need to be developed. Currently, the measurement of triphosphate activity is only available in specific research laboratories. Concentration-controlled therapy seems to be a good method to improve the response to therapy.

Protease Inhibitors

Protease inhibitors (PIs) seem to be the most appropriate candidates for TDM. In contrast to NRTI, they do not require intracellular conversion in its active form. Overall, all have a relatively short half-life[8], are easily quantified in plasma and strong evidence support that drug exposure is related to clinical outcome, toxicity and adherence monitoring.

a) Drug levels and potency

Several studies are in progress linking PI exposure to antiretroviral effect. Some of these trials are discussed below.

In the first place, saquinavir (SQV) plasma levels were monitored in 130 HIV infected patients receiving

SQV-HGC 1200 mg TID plus two NRTIs. Linear regression analysis showed a significant positive relationship between the lowest observed SQV concentration and the decline in plasma HIV-1 RNA after weeks 12, 24, 36 and 48. Patients with an estimated SQV trough concentration above 50 ng/mL had a significant higher likelihood of reaching a sustained decline in HIV-1 RNA above 2 logs below baseline after 48 weeks of therapy[18].

Both a high baseline viral load and pre-treatment with PIs have been proved as the major determinants for virological failure. In an attempt to demonstrate the importance of low indinavir (IDV) levels as predictor of virological failure, 65 patients receiving IDV 800 mg TID as part of a triple regimen were monitored. Univariate analysis demonstrated that a low IDV level (less than 75% of expected values) was highly correlated with treatment failure[19].

In another study, IDV concentrations were measured after patients had been taking an 800 mg TID oral dose. Virologic and pharmacologic characteristics were compared in a subset of 23 patients who were PI-naive before receiving IDV with two NRTIs. In PI-naive subjects, the IDV AUC 8 h was statistically higher in those with undetectable plasma HIV RNA compared to those with detectable HIV-RNA[20].

In an open-label study conducted in antiretroviral naive HIV infected patients exposed to a quadruple drug regimen (NFV + SQV + d4T + 3TC), a significant positive correlation was found between the elimination constant rate and the median NFV or SQV concentration ratio in univariate analysis[21].

In another trial, 18 PI-naive patients receiving a therapeutic regimen including 600 mg twice daily of both RTV and SQV were analyzed. At week 5 (16 patients available), 11 were responders. At week 13, 6 remained responders (two with undetectable viremia). Of note, responders had higher drug levels than non-responders[22].

In the Trilege study, participants received an induction regimen with AZT/3TC/IDV, and were randomized after 3 months to remain on this regimen or reduce to either AZT/3TC or AZT/IDV. A case control study was established to examine the reasons for failure, and RT and protease genotypic resistance assays and IDV drug levels were measured at time of the viral rebound and 6 weeks later. This analysis revealed a striking relationship between drug levels and viral rebound. Among the cases that experienced viral rebound, all those in the triple therapy group had IDV drug levels below the therapeutic range and in 11/13 IDV levels were undetectable, suggesting lack of compliance. In the AZT/IDV group, 10/19 cases had IDV levels below the therapeutic range. Resistance assays showed protease mutations in only one patient at any time point, but M184V was seen in 6/8 cases on triple therapy and in all 25 cases on AZT/3TC. Adherence measured by pill counts showed a significant difference between cases and controls, and correlated with drug levels. When viral load rebound was analysed against IDV drug levels, a strong inverse relationship was seen[23].

Many other studies have found a good correlation between plasma drug concentrations and antiviral effect of antiretroviral therapy[24-27]. Taking into account that there are significant relationships between pharmacokinetic parameters such as AUC, C_{max} and/or C_{min}, and the response to antiretroviral treatment, the maintenance of plasma concentrations above the IC_{95} for both WT and mutant HIV strains, will make ensure treatment success.

The use of genotipic guided therapy seems to have a great value in the design of any optimal antiretroviral regimen. The VIRADAPT study[28] was a prospective randomized study comparing genotype-guided therapeutic decision vs. standard of care in 100 patients on a stable failing antiretroviral regimen. The study demonstrated that genotypic resistance testing improved the clinician's ability to design an effective salvage regimen. A pharmacological substudy was carried out in 87 patients. Plasma trough levels were determined in these patients during the 12-month study period by HPLC. Linear regression analyses showed a statistically significant correlation between plasma concentrations and HIV-RNA for each PI. Higher drug concentrations correlated with lower HIV-RNA levels for the four PI´s. Then, patients enrolled in the study were divided into two groups: 1) optimal concentration (OC) group, which included patients who had trough concentrations less than twice the IC_{95} for each PI on no more than one occasion, and 2) suboptimal concentration (SOC) group, which included patients who had trough concentrations less than twice the IC_{95} on two or more occasions. After 48 weeks of follow-up, patients in the OC group (68%) had a 1.2 log decline in HIV-RNA while patients in the SOC group (32%) had a 0.36 log decline[29-31].

b) Drug levels and toxicity

Toxicity associated to antiretroviral drugs is a common reason to change one or more drugs from the initial prescribed regimen. Recently, various studies have demonstrated a correlation between plasma drug concentration of PIs and more incidences of adverse reactions to antiretroviral therapy.

In a pharmacokinetic substudy of the CHEESE trial[32], patients receiving IDV 800 mg TID (+AZT/3TC) were analysed for the possible relationship between PI plasma levels and the incidence of side effects. Each serum concentration was compared with the average value in a reference population at the same time point after ingestion of IDV. An IDV concentration ratio above 2 was significantly associated with an increase in serum creatinine of more than 30 micromol/L[33].

In another study, 17 patients taking IDV 800 mg TID presented urologic complaints (kidney stones, dysuria, pyelonephritis). They were compared to 14 patients taking the same dose without any urological complications (control group). The mean IDV concentration in patients with urological complaints was significantly higher than in the control group. Urological complications occurring during IDV treatment were associated with elevated IDV plasma concentrations in 80% of patients[34].

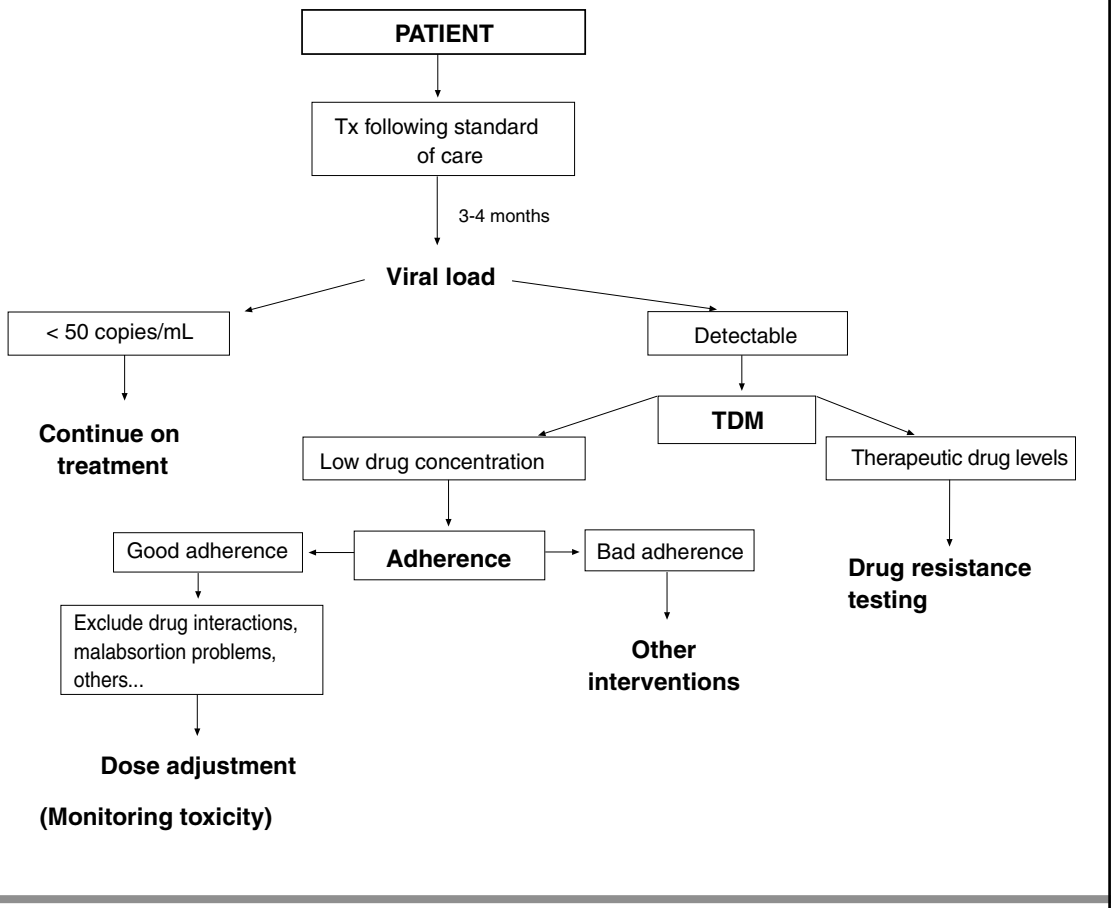

Fig. 1. *Algorithm for using TDM.*

In the ADAM study[21], the toxicity of a quadruple induction regimen was monitored. Plasma concentrations of PIs were assessed. All gastrointestinal complaints, except for diarrhea, were associated with higher plasma levels of SQV or NFV[35].

Peak (C_{max}) and trough (C_{min}) RTV plasma levels were compared in 11 patients experiencing side effects (group A) versus 10 patients without side effects (group B). Both C_{max} and C_{min} were significantly higher in patients complaining of neurological and gastrointestinal side effects[36].

c) Drug levels and adherence

Adherence to antiretroviral therapy has been considered one of the most important predictors of treatment success. Several studies have now shown that nonadherence is an independent risk factor for treatment failure or detectable HIV-1 viremia[4,37-40]. A good compliance allows the maintenance effective and regular plasma drug concentrations. Conversely, as a consequence of "treatment holidays" the evolution of drug resistant HIV-1 strains takes place[41].

In the Trilege study[23], the knowledge of plasma drug concentrations and genotyping testing led to the conclusion that viral failure was firstly not associated with resistance, but resulted from not taking the drugs.

There are no good and reliable methods to assess adherence to antiretroviral therapy. Methods that have been used include the pill count, patient's interview, prescription refill history, and electronic monitoring devices. The best results are obtained by combining several methods. In this context, TDM may provide clinicians with an additional tool to evaluate patient's adherence to the prescribed medications.

To assess the validity of serum PI concentrations as a marker of adherence, compared to self-reported adherence questionnaire (PMAQ) and viral response, trough levels of IDV, NFV, SQV and RTV were measured cromatographically after the last PI dose. Patients were classified as adherent if their serum PI levels were above the reference values (IDV = 70 ng/mL, NFV = 1 μg/mL, SQV = 100 ng/mL, and RTV = 2,1 μg/mL). Appropriate serum levels were significantly associated with viral response and with self-reported adherence [42]. Other studies have confirmed that there is a good correlation between serum drug levels and other methods for assessing compliance to the antiretroviral treatment[43]. The combined use of TDM and drug resistance testing will result in a significant improvement in the management of antiretroviral therapy (Fig. 1).

Recently, some authors have shown that PI concentrations in hair correlate well with HAART res-

ponse. In a prospective study, 132 HIV-infected patients, PI naive and with detectable plasma viremia, started HAART including IDV (n = 90) or SQV (n = 42). Hair IDV levels, in the first 2 cm segment, were higher in responders (R) (n = 66) than in non-responders (NR) (n = 24). IDV levels were also higher in the more distal hair segment of responders. Hair SQV levels were also higher in R (n = 22) than in NR (n = 20). The majority of NR patients with a high hair PI level had strains with several mutations in the protease gene. Among the NR with a low hair PI level, the majority had susceptible strains[44,45]. Thus, the monitoring of hair PI levels allows the detection of poor adherence or absorption problems, and combined with the recognition of mutation at the protease, should facilitate the management of HAART in non-responder patients.

Other studies have shown the possibility of monitoring antiretroviral therapy using other biological fluids such as saliva[46,47]. Saliva concentrations of protease inhibitors may act as easily accessible indicators of plasma levels, and moreover represent the individual free fractions of total concentrations. Saliva concentrations could therefore be employed for TDM.

Non Nucleoside Retrotranscriptase Inhibitors

Available data for nonnucleoside reverse transcriptase inhibitors (NNRTIs) indicate that these drugs are not good candidates for TDM. They have long half-lives and attain high steady steady-state concentrations[8], often several fold higher than the IC_{95} of wild type or even resistant mutant viruses [48,49]. Therefore, it should be unlikely to find low plasma concentrations to explain failure. NNRTIs have a low genetic barrier and failure is almost always due to resistance. However, recent studies have shown a good correlation between steady-state trough concentrations (C_{min}) and response to antiretroviral therapy including NNRTIs.

In an open label phase I/II study, patients received a single 400 mg daily dose of NVP for 24 weeks. Among 10 patients, 8 were responders and 2 were nonresponders. Drug levels in responders were higher than in nonresponders, and the median value in the responders was 4.7 μg/mL, which was significantly higher than in nonresponders (3.1 μg/mL)[50].

In a more recent substudy, data of the INCAS trial[51], it was explored whether there was a relationship between plasma concentrations and efficacy. The analysis showed that a high exposure to NVP was associated with a more rapid initial clearance of HIV-RNA from plasma, a higher chance to reach undetectable plasma HIV-RNA, and a sustained suppression of HIV-1 replication. The plasma drug concentration of NVP that better discriminated between good and poor responders was 3.5 μg/mL[52].

For efavirenz it has also been investigated whether a relationship exists between efficacy and trough concentrations. In 124 patients who received 600 mg of EFV daily and were > 80% compliant (per pill count), treatment failure was more than three times more frequent when trough concentrations were below 3.5 μM (1.1 μg/mL) as compared to those patients with trough concentrations above 3.5 μM (1.1 μg/mL). The relationship between efficacy and trough concentrations in these patients shows the importance of maintaining EFV trough concentrations above 1.1 μg/mL[53].

More studies are necessary to demonstrate that TDM for NNRTIs is useful in clinical practice. In the meantime, rapid, precise, specific and sensitive cromatographically methods have been developed for measuring NNRTIs, and will help to establish the validity of TDM for these drugs[54-56].

Other applications of TDM

TDM has demonstrated its utility in recognizing the possibility of gastrointestinal malabsorption, measuring plasma concentrations of PI[57,58]. In the case of virological failure, pharmacological studies and evaluation of resorption disorders, should be done to find their possible impact on antiretroviral treatment. In patients with malabsorption disorders pharmacological studies can help to adjust the individual dose.

Chronic liver disease is common in HIV-infected patients, mainly due to hepatitis B or C. Drugs extensively metabolized by the liver could increase their plasma levels in the presence of liver malfunction, and TDM has been demonstrated to be helpful in this context[59].

The large number of drugs used in HIV infection produce a great number of potentially drug-drug interactions, which can compromise antiretroviral treatment. In particular, the interaction of PIs with the CYP450, which is the metabolic way for many other drugs, can lead to suboptimal or excessive drug concentrations. TDM of PIs may be useful to maintain therapeutic drug levels and avoid adverse reactions related to excessive drug concentrations.

Limitations of TDM

A high baseline viral load, a low CD4 count, previous antiretroviral treatment, and the use of antiretroviral drugs with a low bioavailability as SQV HGC have all been related to treatment failure in most studies. In the end, the outgrowth of resistant mutant HIV is the consequence of incomplete viral suppression[7]. The maintenance of higher drug concentrations of antiretroviral agents is really required to effectively block replication (IC_{95}) of both wild type and mutant strains. However, the knowledge of the IC_{95} for PIs requires a reliable estimation of their *in vivo* potency as well as an accurate measurement of their pharmacokinetic profile in humans.

Protein binding

The distribution of drugs from plasma to tissues depends on the free drug concentration and hence is a function of the proportion of drug coupled to

serum proteins. *In vitro* assays not including human serum proteins may overestimate the *in vivo* activity of PIs and other drugs, like EFV, which are highly protein bound.

The most important proteins in plasma are serum albumin (HSA), α-1 acid glycoprotein (AGP), and lipoproteins (LIPOs). HSA is the most abundant protein in plasma (40 mg/mL), whereas normal AGP plasma level varies between 0.5-1 mg/mL. *In vitro* assays used to determine the potency of antiretroviral drugs which do not consider normal protein concentrations in the cultured medium. Thus, the results reflect antiviral potency in tissue culture medium containing 5 to 10% fetal calf serums. However, PIs (except IDV) and EFV binds to components of human plasma into a significant degree. Several authors have studied the influence of an increase in serum protein concentrations on cultured medium on the potency of antiretroviral drugs. Molla *et al.*[60] assayed the *in vitro* activity of PIs against wild type and mutant HIV isolates in the presence of human serum (HS 50%). Increasing concentrations of HS significantly affected the antiviral activity of RTV. Adding 50% of HS, the IC_{50} was 20-fold higher than observed with 0% HS. Similarly, the average potency of SQV and NFV was markedly attenuated (25- and 35-fold, respectively) in the presence of serum. In contrast, the IC50 of IDV was nearly unaffected by serum binding (2-fold average change in IC_{50}), and the activity of amprenavir was only modestly (6-fold) affected. The authors further examined the activity of PIs against viral clones containing specific mutations into the HIV genome. The median fold difference in IC_{50} between 0 and 50% HS for the 10 mutant viruses for each inhibitor was the following: RTV 24; SQV 33; IDV 1.8; NFV 38; and amprenavir 8.7. These results suggest that plasma protein binding significantly attenuates the antiviral potency of RTV, SQV and NFV *in vivo.* In contrast, the potency of IDV may be more accurately represented by *in vitro* virological assays in the absence of HS.

In another study, the effect of AGP on the antiviral activity of PI's was determined by calculating the percentage reduction in p24 generation by PIs in the absence or presence of 0.5 mg/mL and 2 mg/mL AGP. AGPs are acute-phase proteins that can increase in plasma as much as 5-fold during acute and chronic infections and inflammation. In HIV infection, AGP concentration can be elevated in the setting of uncontrolled HIV replication and/or opportunistic infections, which can reverse upon treatment. For numerous basic drugs, the concentration of AGP in the plasma inversely correlates with free drug concentration available for activity. AGP concentration of 0.5 mg/mL is the mean concentration found in normal subjects; 2 mg/mL is the mean concentration observed during acute or chronic infections. The effect of a 4-fold increase in α1-acid glycoprotein on the individual efficacy of 5 PIs was in PBMC infected with protease WT and PI-resistant HIV isolates. For wild type virus, the efficacy of the PIs at trough concentrations was unaffected by a 4-fold increase in AGP. However, for the partially HIV PI-resistant isolate, a 4-fold increase in AGP resulted in 2, 30, 37, 37 and 42% loss of activity for IDV, SQV, NFV, RTV and amprenavir, respectively. The high-level HIV PI-resistant isolate had a greater loss in activity. The change in IC_{50} secondary to the addition of AGP was greater for RTV, NFV, and amprenavir (above 10-fold), and lower for IDV[61].

The impact of human plasma proteins on the potency of EFV was assessed by conducting potency assays in MT2 cells in the presence or absence of 45 mg/mL human serum albumin plus 1.0 mg/mL α1-acid glycoprotein. Much higher concentrations (16.5-fold above) were required to achieve 90% inhibition of HIV-1 replication[62]. Therefore, the IC_{90} for drugs with a high protein binding must be adjusted. *In vitro* assays to estimate the IC_{90} for antiretroviral drugs must be done in tissue culture experiments with added human serum albumin and α1-acid glycoprotein at concentrations mimicking those present in AIDS patients. Moreover, we must take into consideration that AGP is an inducible acute phase reactant protein, in order to estimate the real plasma drug concentration of the drug. But even knowing the plasma drug concentration required to obtain an effective free drug concentration, its penetration in some compartments might be compromised.

P-glycoprotein

The overexpression of the multidrug-resistance transporter P-glycoprotein (P-gp) has been proposed recently as one of the mechanisms that could limit the absorption, brain entry, and the penetration into lymphocytes and macrophages of some drugs. This fact might prompt the emergence of resistance mutants, and allow continued viral replication[63, 64]. P-gp is an ATP-dependent efflux pump typically associated with multidrug resistance in cancer chemotherapy. P-gp mediated efflux reduces the intracellular accumulation of these compounds, diminishing drug efficacy[65]. It is expressed in the epithelial cells of the GI tract, kidney, liver, and blood brain barrier. SQV, RTV, NFV and IDV were recently shown to interact with the multidrug-resistant protein in the human intestinal epithelial cell line CaCO2[66], and a human carcinoma line, KB-V1[67]. More recently, the importance of P-gp mediated efflux has been demonstrated, too, in the bowel[68]. Expression of P-gp results in reduced drug absorption on the gastrointestinal tract, and enhanced elimination into the bile and urine. On the other hand, P-gp is a critical component of the blood-brain barrier, which prevents to the access of systemic drugs to the central nervous system. The presence of a barrier to the drug's distribution into the brain caused by P-gp suggests that the ability to reach therapeutic brain concentrations is limited, creating a potential sanctuary for viral replication. P-gp and other multiple drug resistance-associated proteins, such MRP1[69], are also found on the surface of lymphocytes (including about 10% of CD4+ cells)[70] and macrophages, which are major targets for HIV-1 infection. A study showed that both H9

(T cell line) and U937 (monocityc cell line) cells, upon infection with HIV, express increased levels of P-gp and accumulated significantly less AZT as compared to uninfected cells[71]. Therefore, AZT and other nucleoside analogues are substrates for P-gp mediated efflux[72].

In another study[73] in which peripheral blood CD4+ and CD8+T cells from 16 patients with HIV-1 infection, 8 each with CD4+ counts above (group I) and below (group II) 200 cells/mm^3 were examined for the expression of P-gp. A significantly increased proportion of CD4+ T cells from patients with HIV-1 infection expressed P-gp as compared to controls, resulting in an increase in the ratio of CD4+P-gp+/ CD8+Pgp+ cells. This ratio in group II patients was significantly higher than in group I patients, suggesting a progressive increase in P-gp expression on CD4+ cells with the advancement of HIV-1 infection. Therefore, an increased expression of P-gp might be an additional mechanism leading to resistance to nucleoside analogues. Of interest, the overexpression of P-gp seems not to be induced by the administration of NRTIs [74], although overall increases as HIV disease progresses.

Along with CYP3A-mediated metabolism by enterocytes, P-gp may limit the oral bioavailability of PIs by transporting the absorbed drug back into the intestinal lumen. The expression of P-gp on the blood brain barrier and on the surface of lymphocytes and macrophages may result in an incomplete viral suppression in these compartments, which act as potential sanctuaries far from drug access. Thus, low baseline CD4+ counts and advanced stages of HIV infection are associated with an increased expression of P-gp, resulting in low penetration of PIs to these compartments. In this situation, the measurement of intracellular drug concentrations may provide a better correlation with the level of virologic suppression. Up to now, no reports have proven an interaction between NNRTIs and P-gp or another multiple-drug resistance associated protein.

Variability in pharmacokinetics

Intrapatient variability is postulated to be one of the limitations that makes the use of TDM unreliable in the management of antiretroviral therapy. Variability during various phases of the menstrual cycle has been described for ZDV and IDV[75]. These findings suggest that TDM is limited into a great extent, and that concentrations measured on one day may not be the same another day. The implications of these findings on TDM must be carefully evaluated.

TDM in antiretroviral therapy requires a wide knowledge of drug pharmacokinetics and their interactions. For instance, food interactions seem to play an important role because they can modify the C_{max}, AUC, C_{min} and T_{max} of any antiretroviral compounds. For example, food delays the T_{max} for NFV and the concentration obtained before dosing might not be the lowest point in the dosage interval. Finally, sample timing is particularly important. The election of a wrong timing could lead to a non representative sample.

Conclusions

The introduction of TDM represents a major step in the way to optimize antiretroviral therapy for each individual. Firstly, it can improve the response to treatment by just modifying drug doses. There is no doubt that the integrated use of TDM and drug resistance testing will result in a significant improvement in the management of antiretroviral therapy.

Secondly, an important application of TDM concerns the adherence issue. This aim should be used cautiously because serum concentrations may reflect drugs administered within the past 24 hours but say nothing about medications taken days or weeks before blood sample collection. Monitoring hair levels has been proposed as a good method to assess past adherence based on the continued growth of the hair and the accumulation of the drug in this tissue.

Thirdly, TDM also seems to be of great utility to detect drug-drug and drug-food interactions and malabsorption problems, and could allow dose adjustment in order to maintain effective plasma concentrations. However, more studies are necessary to determine a therapeutic range in which plasma drug concentrations need to be kept. The recognition of toxic and suboptimal drug concentrations needs to be established for each compound. Controlled-concentration therapy seems to be the most appropriate strategy to maintain serum drug concentrations much higher than the IC_{95} for each drug. There is a need for a reliable estimation of the IC_{90} for *wild type* as well as mutant HIV strains.

Fourthly, PIs and NNRTIs are the most appropriate candidates for TDM. The applicability is still unclear for NRTIs, since they need to be intracellularly phosphorilated to become active.

Finally, the additional costs of TDM as part of clinical routine exams need to be balanced against an expected reduction in pharmaceutical costs. Dose individualization needs to be pursued in each individual for providing an effective drug concentration. This represents a more rational use of antiretroviral drugs and might prevent therapeutic failure due to low plasma drug concentrations.

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