Pharmacokinetic Investigation of Imatinib Using Accelerator Mass Spectrometry in Patients with Chronic Myeloid Leukemia


Abstract

Purpose: To investigate the potential use of accelerator mass spectrometry (AMS) in the study of the clinical pharmacology of imatinib.

Experimental Design: Six patients who were receiving imatinib (400 mg/d) as part of their ongoing treatment for chronic myeloid leukemia (CML) received a dose containing a trace quantity (13.6 kBq) of $^{14}$C-imatinib. Blood samples were collected from patients before and at various times up to 72 h after administration of the test dose and were processed to provide samples of plasma and peripheral blood lymphocytes (PBL). Samples were analyzed by AMS, with chromatographic separation of parent compound from metabolites. In addition, plasma samples were analyzed by liquid chromatography/mass spectrometry (LCMS).

Results: Analysis of the AMS data indicated that imatinib was rapidly absorbed and could be detected in plasma up to 72 h after administration. Imatinib was also detectable in PBL at 24 h after administration of the $^{14}$C-labeled dose. Comparison of plasma concentrations determined by AMS with those derived by LCMS analysis gave similar average estimates of area under plasma concentration time curve (26 ± 3 versus 27 ± 11 μg/mL-h), but with some variation within each individual.

Conclusions: Using this technique, data were obtained in a small number of patients on the pharmacokinetics of a single dose of imatinib in the context of chronic dosing, which could shed light on possible pharmacologic causes of resistance to imatinib in CML.

Imatinib, an inhibitor of the Bcr-Abl tyrosine kinase, is an effective treatment for chronic myeloid leukemia (CML) and has revolutionized the treatment of this disease (1). However, some patients fail to achieve a complete hemato logical or molecular response, and others relapse with disease that is then resistant to treatment with imatinib (2, 3). A number of theories have been advanced to account for CML resistance to imatinib, including innate or acquired mutations in the Bcr-Abl gene (4, 5), imatinib binding to α₁-acid glycoprotein (6, 7), and altered imatinib pharmacokinetics (8–10). The possibility that imatinib resistance is due to initial or acquired differences in drug absorption, distribution, or metabolism has been explored to some extent in conventional pharmacokinetic studies (9, 11–13). Using liquid chromatography/mass spectrometry (LCMS) to measure drug and metabolites in plasma (12, 13) or using $^{14}$C-imatinib to detect total drug-derived species in mass-balance studies (14), a number of metabolites of imatinib have been identified. The major metabolite is CGP74588, which accounts for ~10% of an oral dose of imatinib and retains inhibitory activity towards the Bcr-Abl kinase (13).

The bioavailability of imatinib is close to 100% from either tablets or capsules and does not seem to vary significantly among individuals, during chronic therapy or with the dose administered (15, 16). Therefore, the most likely changes in pharmacokinetics that may influence therapeutic efficacy are in metabolism or distribution, given that only a small fraction of a dose of imatinib is excreted as parent drug in the urine. Analysis based on LCMS may not be sufficiently sensitive in all cases to detect altered metabolism, binding to plasma proteins, or distribution to the target tissue in the relevant patient population.

Accelerator mass spectrometry (AMS) is a relatively novel technique that can be used to measure extremely low concentrations of drug and metabolites in a variety of tissues (17, 18). AMS has previously been used in mass-balance studies (19) and in micro-dosing studies in early drug development.
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To assess the suitability of AMS as a technique to evaluate the pharmacology of imatinib in patients on long-term therapy for CML, a pilot study in six patients was done. Plasma and peripheral blood lymphocyte samples were obtained during a 72-h period after administration of a $^{14}$C-labeled dose of imatinib. Data from the AMS analysis were compared with conventional LCMS data on imatinib and its major metabolite.

**Materials and Methods**

Capsules containing 100 mg of $^{14}$C-imatinib as the mesylate salt at a specific activity of 135.7 Bq/mg were supplied by Novartis AG. The structure of imatinib, indicating the position of the $^{14}$C label (‘), is shown in Fig. 1. The metabolite CGP74588 results from demethylation of the piperidine ring, and so retains the radiolabel.

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samples were centrifuged at 15,000 g. The mobile phase consisted of 60% acetonitrile and 40% water for the high-performance liquid chromatography separation. Twenty microliters of plasma were injected onto the LCMS. The lower limits of quantitation for unchanged imatinib detected by AMS following high-performance liquid chromatography separation was 380 pg/mL (0.003 dpm/mL), and that for total 14C was equivalent to 6 ng/mL (0.06 dpm/mL). The coefficient of variation for the AMS assay was >5% across the range of concentrations analyzed.

A separate aliquot of plasma was analyzed by a previously published LCMS method for imatinib and the major metabolite CGP74588 (22). To 200 μL of plasma, 50 μL of methanol and 50 μL of internal standard solution (540 ng/mL D8-imatinib) were added, followed by 250 μL of acetonitrile and vortex mixing for ~20 s. The samples were centrifuged at 15,000 × g for 5 min in a microfuge and 150 μL of the supernatant were transferred to a limited volume high-performance liquid chromatography insert. Twenty microcillers were injected onto the LCMS. The mobile phase consisted of 60% methanol, 40% 0.02 mol/L ammonium acetate at a flow rate of 300 μL/min. Analysis was done on an Applied Biosystems API2000 in MRM mode, with parent/daughter ion combinations of m/z 495/395, 481/395, and 503/395 for imatinib, CGP74588, and the D8 internal standard, respectively. The lower limits of quantitation for parent drug and metabolite were 1 and 2 ng/mL, respectively. Plasma concentrations of α1-acid glycoprotein were determined according to a previously published method (23).

### Results

Patients’ ages ranged from 35 to 54 years; duration of imatinib treatment was 20 to 63 months; and time since diagnosis ranged from 31 to 71 months (Table 1). There were no adverse effects observed in patients participating in this investigation. Data from patient 1 were excluded from some elements of the summary in Table 2 because he had taken his subsequent dose before the 24-h sample was drawn. This dose did not contain 14C-imatinib and, hence, did not affect the AMS analysis.

A plot of the uncorrected LCMS data for imatinib, CGP74588, and total 14C material by AMS is shown for patient 5 in Fig. 2. Apart from patient 1, in whom the concentrations of imatinib and CGP74588 as measured by LCMS increased between 8 and 24 h, and patient 6, who had a long half-life (33 h) for 14C-imatinib, the estimates of terminal half-life from either LCMS or AMS data were within 1 h of each other in all patients. Figure 3 shows the corrected data for patient 5 after subtracting the imatinib concentrations predicted to be present from previous imatinib doses, based on the LCMS data. In this patient, there was good agreement between the imatinib concentrations as determined by LCMS and AMS, with the imatinib AUC0–∞ corrected for prior dose within 2% of the value generated from the LCMS data.
To compare the known imatinib-derived materials to the total $^{14}$C material measured by AMS, concentrations of imatinib and CGP74588 as determined by LCMS, were combined. The contribution from previous doses of imatinib to this composite material was subtracted for comparison with the AMS data. No correction for the change in molecular weight was applied because the $^{14}$C measure represents a mixture of contributions from species of varying molecular weight. A comparison of total $^{14}$C material by AMS and the sum of imatinib and CGP74588 is shown for patient 5 in Fig. 3.

Applying noncompartmental analysis to the AMS and LCMS data, Fig. 4 shows a comparison of AUC$_{0-24}$ for the single dose of imatinib measured by two different methods (LCMS and AMS) and AUC$_{0-24}$ for each of the six patients. There was poor agreement between AUC$_{0-24}$ (41 ± 13 µg/mL h) for the uncorrected LCMS data and AUC$_{0-24}$ for the LCMS method for all six patients. Where comparisons have been made with steady-state conditions, the AUC values in the present study may involve an accuracy in the calculation of half-life from the LCMS data, which may account in part for the discrepancy between single-dose and steady-state AUCs. Previously published data on the use of radiolabeled drug to investigate the pharmacokinetics of a single dose at steady-state are very limited (27). It may be that the pharmacokinetics of the parent drug alone does not give sufficient information to identify the effects of enzyme induction or qualitative changes in drug metabolism.

Previous pharmacokinetic studies have focused largely on the pharmacokinetics of imatinib and CGP74588 after single oral doses (26). Where comparisons have been made with steady-state pharmacokinetics, a modest degree of accumulation has been observed, together with a slightly longer half-life at steady-state (28). Pharmacokinetic variables in the current study, including $C_{\text{max}}$ (2.7 ± 0.9 µg/mL), $T_{\text{max}}$ (3 ± 2 h), and half-life (21 ± 6 h), derived from the LCMS analysis of imatinib (Table 2) were similar to those previously reported (11, 26, 28), although the half-life value is slightly higher than that reported by Peng et al. of 19.3 h.

In a study using $^{14}$C-imatinib (14), healthy volunteers received a single oral dose of 200 mg of imatinib with a total concentration of $^{14}$C-labeled drug (137 Bq/mg).

**Discussion**

The emergence of resistance to imatinib, particularly in CML, may be seen as inevitable, and the identification of mutations in Bcr-Abl (4) fits the model of resistance due to selective pressure on a genetically unstable or heterogeneous population of tumor cells. However, there are a number of studies that have suggested that pharmacokinetic explanations may underlie innate or acquired resistance to imatinib in some patients (7–10). One approach to overcoming such variability is to use doses higher than 400 mg/d (24, 25).

Whereas differences in pharmacokinetic variables following a single dose and at steady-state seem to be modest for the parent drug (26), it has not previously been possible to investigate the pharmacokinetics of an individual dose under steady-state conditions. The AUC values in the present study may involve an inaccuracy in the calculation of half-life from the LCMS data, which may account in part for the discrepancy between single-dose and steady-state AUCs. Previously published data on the use of radiolabeled drug to investigate the pharmacokinetics of a single dose at steady-state are very limited (27). It may be that the pharmacokinetics of the parent drug alone does not give sufficient information to identify the effects of enzyme induction or qualitative changes in drug metabolism.
radioactive dose of 1.18 MBq (32 μCi), 80-fold higher than the dose of radioactivity administered in the current study. In the latter, the AUC_{0-24} was 11 ± 1 μg·mL⁻¹·h and that for CGP74588 was 1.7 ± 0.2 μg·mL⁻¹·h. The AUC_{0-∞} for total radioactivity was 26 ± 1 μg·mL⁻¹·h in plasma. The comparison between imatinib AUC and that for total radioactivity in the current study gives AUC_{0-∞} values of 26 ± 3 and 76 ± 19 μg·mL⁻¹·h. Thus, the ratio between imatinib and total radioactivity is 41% in the previous single-dose study and 34% in the current study at steady state. Albeit in a study with a small number of patients, these data may suggest that the greater proportion of 14C label present as non–parent drug material in the steady-state setting represents a shift towards the formation of other unknown metabolites. By sampling over 1 week following administration, the previous investigation identified a terminal half-life for total 14C material of 57 ± 12 h, compared with a half-life of 32 ± 7 h observed here with sampling out to only 72 h. Previous studies (28) at steady state have reported an AUC_{0-∞} of 82 ± 45 μg·mL⁻¹·h, comparable to that of 72 ± 29 μg·mL⁻¹·h, as measured by LCMS in the current study. Indeed, the values for \( t_{1/2} \) and AUC_{0-24} (Table 2) are almost identical to those previously reported (28).

A potential role for pharmacokinetic variability in resistance to imatinib is supported by evidence from drug binding and transport studies. Binding of imatinib to α1-acid glycoprotein has been shown to limit the antitumor effect of imatinib, an effect which is reversible by displacing imatinib from α1-acid glycoprotein (6). Imatinib has also been identified as a substrate for transport proteins such as ABCB1 (29) and ABCG2 (8). The influence of these proteins on the intestinal absorption and subsequent tissue distribution of imatinib has been suggested to play a role in pharmacologic resistance to the drug (6, 9). The development of resistance during chronic therapy is possibly linked to induction of α1-acid glycoprotein (6) or to changes in imatinib pharmacokinetics over time (30). With regard to binding to α1-acid glycoprotein, it is possible that imatinib and CGP74588 compete for binding sites, such that the fraction unbound in plasma will be affected both by changing concentrations of α1-acid glycoprotein in plasma and by changes in the relative concentrations of drug and metabolite (31). In the patients studied here, levels of α1-acid glycoprotein were not markedly elevated, with little variation among patients. Variation in distribution to cellular components of the blood may also affect drug action, but in the current study the 11C label was detectable in WBC at concentrations close to the limit of detection, and in only three of six patients.

This study has shown that AMS can be applied to investigate the pharmacokinetics of imatinib during chronic therapy. There were some discrepancies between the LCMS and AMS data for parent drug, but only a small number of patients were investigated in this feasibility study. Further analyses, including those of unbound drug and of other potential metabolites, may yield additional insights into the underlying mechanisms responsible for the discrepancies observed in some patients between imatinib concentrations measured by LCMS and AMS. Although there was not a clear association between imatinib pharmacokinetics and clinical response in this small patient group, application of AMS technology in a larger study would be of value in defining the role of pharmacologic variation in resistance to imatinib therapy.

**References**

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