Cancer Chemother Pharmacol (2004) 53: 313–323
DOI 10.1007/s00280-003-0741-6

ORIGINAL ARTICLE

**Philipp le Coutre · Karl-Anton Kreuzer · Stefan Pursche
Malte v. Bonin · Traugott Leopold · Gökben Baskaynak
Bernd Dörken · Gerhard Ehninger · Oliver Ottmann
Andreas Jenke · Martin Bornhäuser · Eberhard Schleyer**

Pharmacokinetics and cellular uptake of imatinib and its main metabolite CGP74588

Received: 13 May 2003 / Accepted: 28 October 2003 / Published online: 5 December 2003

Abstract Despite the remarkable clinical response rates to imatinib in the treatment of bcr-abl leukemic patients, pharmacokinetic data on this relatively novel substance are needed to improve our understanding of the emergence of resistance, the interindividual variations of clinical response and the clinical and biologic relevance of its main metabolite N-desmethyl-imatinib. We present here pharmacokinetic data obtained with a newly designed HPLC approach in 97 patients with chronic myeloid leukemia or acute lymphatic leukemia (ALL) under treatment with imatinib that allowed us to calculate the AUC (39.5 μg·h/ml for an oral dose of 400 mg daily), the $t_{1/2}$ (18.2 h) and the peak concentration (1.92 μ/ml for an oral dose of 400 mg daily) of imatinib in plasma. In a subgroup of patients, the same parameters were analyzed for N-desmethyl-imatinib. We also provide data on the imatinib concentration in the cerebrospinal fluid (CSF) of ALL patients and demonstrate that oral administration of imatinib resulted only in a marginal flux across the blood-brain barrier. Finally, in an in vitro setting, we determined cellular concentrations of imatinib in HL-60 cells and showed an over-proportional uptake both in RPMI medium and in human plasma. Using an arithmetical approach combining all parameters obtained in imatinib-treated patients, we finally provide a conclusive approximation of basic pharmacokinetic data for both imatinib and its main metabolite N-desmethyl-imatinib.

Keywords Imatinib · Metabolite · CGP74588 · N-Desmethyl-imatinib · Pharmacokinetics · CML · ALL

P. le Coutre · K.-A. Kreuzer · G. Baskaynak · B. Dörken
Medizinische Klinik für Hämatologie und Onkologie,
Campus Virchow, Charité, Humboldt Universität Berlin, Germany

S. Pursche · M. v. Bonin · T. Leopold · G. Ehninger
A. Jenke · M. Bornhäuser · E. Schleyer
Universitätsklinikum Carl Gustav Carus,
Abteilung für Hämatologie und Onkologie, Dresden, Germany

O. Ottmann
Medizinische Klinik III, Johann Wolfgang Goethe Universität,
Frankfurt a.M., Germany

E. Schleyer (✉)
Medizinische Klinik und Poliklinik I an der,
Technischen Universität Dresden,
Fetscherstr. 48, 01307 Dresden, Germany
E-mail: schleyer@mk1.med.tu-dresden.de
Tel.: +49-351-4585617
Fax: +49-351-4585362

Introduction

The reciprocal t(9;22)(q34;q11) translocation known as the Philadelphia chromosome is the causative chromosomal abnormality in the pathogenesis of more than 95% of all cases of chronic myeloid leukemia (CML) and is also detectable in some cases of acute lymphatic leukemia (ALL). At the molecular level, the Philadelphia chromosome generates the bcr-abl fusion transcript that results in the expression of the oncogenic bcr-abl protein tyrosine kinase. Previous studies have demonstrated that the constitutive tyrosine kinase activity of this protein is the crucial event in the genesis of bcr/abl$^+$ CML and ALL that leads to blocking of apoptosis and ultimately results in the specific leukemic phenotype.

Imatinib mesylate (Glivec, Gleevec, formerly known as STI571) represents a novel treatment modality that is active through selective inhibition of the ATP-binding pocket present in the abl domain of the bcr-abl tyrosine kinase [1, 2]. Consequently, imatinib results in continuous inactivation of bcr-abl tyrosine kinase activity and eradicates the leukemic phenotype by reconstitution of apoptosis in bcr-abl$^+$ cells [3, 4, 5]. Previous clinical trials in patients with chronic-phase disease who have failed interferon-α treatment have demonstrated remarkable hematologic and cytogenetic response rates [6]. A currently ongoing phase III clinical trial has demonstrated the superiority of imatinib over interferon-α in inducing a cytogenetic response within the first 2 years of treatment in patients with chronic-phase

disease and in an overall survival advantage in the first year of treatment in combination with fewer grade 3/4 adverse events [7]. However, long-term results are still lacking. Hematologic and cytogenetic remissions are also present in patients with accelerated phase and blast crisis [8, 9].

Chemically, imatinib is 4-[(4-methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamide methansulfonate ($C_{30}H_{35}N_7SO_4$). In the clinical setting, imatinib is administered orally at a dose of 400 mg daily in patients with chronic phase disease and at 600 mg daily in patients with accelerated phase and blast crisis disease including dose modifications according to adverse events and hematologic response. Despite the increasing number of reports on the clinical effects of imatinib, only few focus on the pharmacokinetic characteristics of imatinib in humans. Moreover, no data about cellular concentrations and uptake of imatinib are available.

However, profound pharmacokinetic data will be essential to answer questions related to clinical aspects of imatinib therapy such as passage through the blood-brain barrier, the elimination of imatinib in patients with acute or chronic renal failure as well as interindividual variation of peak plasma levels. Further, previous studies have shown that potential inactivation of imatinib through excessive binding to the plasma protein α1-acid glycoprotein may result in decreased levels of active drug which consequently may result in the induction of resistance [10, 11, 12, 13].

According to the manufacturer, the mean absolute bioavailability of imatinib when administered as capsules is 98%. About 13% is excreted in the urine, but the main site of degradation is the liver. Here, imatinib is a substrate of the cytochrome P 450 isoenzyme cytochrome P3A4 (CYP3A4). The main metabolite of imatinib after metabolization by CYP3A4 is CGP74588 ($C_{29}H_{33}N_7SO_4$) also called N-desmethyl-imatinib. N-desmethyl-imatinib, according to the manufacturer, shows comparable biologic activity regarding the bcr-abl, c-abl, PDGF-R and c-kit tyrosine kinases [14]. However, no data are available that compare imatinib and N-desmethyl-imatinib in an in-vivo setting.

Here, we provide data on the pharmacokinetics and cellular uptake of imatinib and its main metabolite obtained by a newly developed high-performance liquid chromatography (HPLC) assay in 39 patients with CML under treatment with 400, 600 and 800 mg daily. Additionally, we analyzed cerebrospinal fluid (CSF) concentrations of imatinib in 17 patients and 24-h urine elimination of imatinib and N-desmethyl-imatinib in another 7 patients. Moreover, in 58 bcr/abl$^+$ ALL patients, we analyzed on a single sample basis imatinib and N-desmethyl-imatinib concentration ratios. In two CML patients, imatinib and N-desmethyl-imatinib were determined after discontinuation of imatinib prior to allogeneic peripheral stem cell transplantation. Data on the cellular uptake of imatinib and N-desmethyl-imatinib investigated in HL-60 cells in vitro are also presented.

Material and methods

Patients

A total of 97 patients were analyzed in this study. Of these patients 39 (40.2%) had bcr/abl$^+$ CML and 58 (59.8%) bcr/abl$^+$ ALL. Table 1 summarizes all analyses undertaken in individual patients.

The plasma samples from the first subgroup of the study were derived from 32 CML patients who were treated in at least one of our centers as part of an advanced access program with imatinib (CSTI571 113-115). Plasma samples from 12 of these patients were available over a time period of more than 2 days. Six of these patients were in chronic phase and consequently were treated with 400 mg imatinib daily. The other six patients were in either accelerated phase or blast crisis and were treated with 600 mg imatinib daily. From the remaining 20 CML patients at least three samples per patient were available and analyzed in an average calculation of all data points. All patients from this subgroup were analyzed after the first administration of imatinib but not under pseudosteady-state conditions.

Blood samples from this group were taken before oral intake of imatinib and at various time-points (0.5, 1, 3 and 6, 12, 24, 24.5, 25 and 27 h etc. up to 4 days) thereafter. Samples were then centrifuged and plasma was stored at −20°C pending analysis. Since the HPLC analysis at this time was not validated for the measurement of N-desmethyl-imatinib, the results from this patient subgroup are exclusively imatinib pharmacokinetic parameters. After the validation of the HPLC method for analysis of N-desmethyl-imatinib (see below), the analysis of all samples in the remaining patient subgroups included measurement of N-desmethyl-imatinib.

Table 1 Analyses undertaken in the individual patients

Patient nos.	Diagnosis	Imatinib dose (mg)	Plasma analysis >24 h	Single-day plasma analysis	Metabolite analysis	CSF analysis	Urine analysis	Analysis at steady-state
1–6	CML	400	Yes	No	No	No	No	No
7–12	CML	600	Yes	No	No	No	No	No
13–21	CML	400	No	Yes	No	No	No	No
22–32	CML	600	No	Yes	No	No	No	No
33–72	ALL	400/600	No	Yes	Yes	No	No	Yes
73–86[a]	ALL	400/600	No	Yes	Yes	Yes	No	Yes
87–89[b]	ALL	400/600	No	Yes	Yes	Yes	No	Yes
90–94	CML	600	No	Yes	Yes	No	Yes	Yes
95, 96	CML	800/500	Yes	No	Yes	No	Yes	Yes

[a]Patients without meningeosis leukemia
[b]Patients with meningeosis leukemia

The second subgroup comprised 56 ALL patients who were treated as part of a randomized, multicenter phase II trial in elderly bcr/abl$^+$ ALL patients (GMALL-STI571-Elderly-01/02). In this group 17 CSF samples were analyzed under pseudosteady-state conditions and single point plasma sample analyses were performed in all individuals.

Finally, a third group comprised seven CML patients in whom 24-h urine elimination was determined and the decrease in plasma imatinib and N-desmethyl-imatinib concentrations were determined after discontinuation of imatinib prior to peripheral blood stem cell transplantation.

Cellular uptake

Cellular uptake of imatinib was investigated in HL-60 cells incubated in RPMI medium or human plasma at concentrations ranging from 150 ng/ml to 50 μg/ml. In total, three independent experiments over a time range of 6 weeks were performed. All measurements were done in triplicate. In addition to the intracellular imatinib analyses, the concentrations in the incubation medium (RPMI and human plasma) were determined at the starting point and at the endpoint in order to validate the analysis. Also all washing solutions were analyzed for imatinib residues. As expected, no metabolism of imatinib by HL-60 cells was found (data not shown). Therefore, the incubation system could be defined as a ''closed system'' and the sum of the imatinib in all compartments should amount to 100% in relation to the starting concentration. Preliminary experiments focused on the distribution of imatinib within the various compartments of this system. In fact, we found that the sum of all amounts of imatinib in all compartments ranged from 75% to 125%, probably reflecting measurement variations as well as the surface activity of imatinib in aqueous solution (data not shown). We also determined the rediffusion of intracellular imatinib to the washing solution and found that about 10% of the intracellular imatinib was redistributed within 5 min to the surrounding medium (data not shown). Based on this observation the centrifugation time for the washing step was exactly 5 min in each experiment.

For each cellular sample, 2×10^7 cells were incubated in 5 ml of either RPMI or human plasma at various concentrations of imatinib as indicated above. After an incubation time of 5 h at 37°C in an atmosphere containing 5% CO_2, the cells were centrifuged at 1000 *g* and the resulting pellets were dissolved in 50 ml isotonic NaCl and subsequently again pelleted by centrifugation. After discharging the supernatant the sample pellets were treated for imatinib extraction similar to plasma (see below).

To determine the cellular uptake of N-desmethyl-imatinib we extracted and purified N-desmethyl-imatinib by liquid–liquid extraction from patient urine and subsequently purified it by the newly developed HPLC method. The resulting N-desmethyl-imatinib solution showed a purity of 99% as tested by analytical HPLC. N-desmethyl-imatinib authenticity was proved by the similarity of UV spectra and existed solely in the plasma or urine of imatinib-treated patients. Finally, N-desmethyl-imatinib authenticity was additionally proved by magnetic resonance spectroscopy (data not shown). Due to the restricted availability of N-desmethyl-imatinib, uptake analysis in HL-60 cells was only performed on a smaller incubation concentration scale than imatinib and solely in RPMI medium.

HPLC and sample preparation

The newly developed HPLC method for analyzing imatinib and N-desmethyl-imatinib will be described in full elsewhere. Briefly, the chromatographic system comprised two Knauer 64 analytical HPLC pumps (Knauer Corporation, Berlin, Germany), a Shimadzu UV spectrometric detector SPD-SA (Shimadzu, Duisburg, Germany), a Waters WISP 712 autoinjector and an electric motor-driven autoswitch equipped with a rheodyne valve 7740-001 for online enrichment switching (Besta-HPLC-Technik, Wilhelmsfeld, Germany). Recording, evaluation and quantitation of chromatograms was done by a PC supported the GINA program from Raytest (Straubenhardt, Germany).

A ZirChrom analytical HPLC column (3 μm, PDB-ZrO_2, 3% carbon, 50×4×6 mm) with a precolumn of the same solid phase specificity was used. The system was designed as an online enrichment system with another PDB-ZrO_2 precolumn as enrichment column. Flow was set at 0.4 ml/min at room temperature for the analytical part and at 2 ml/min at room temperature for the enrichment part. The analytical eluent consisted of 600 ml 0.01 *M* KH_2PO_4/0.09 *M* K_2HPO_4 + 400 ml methanol, while the enrichment eluent consisted of 450 ml 0.1 *M* KH_2PO_4 + 350 ml H_2O + 200 ml CH_3OH. For quantitation the external standard method was used by regression analysis of six spiked plasma samples with 10 ng/ml, 100 ng/ml, 500 ng/ml, 1000 ng/ml, 10 μg/ml, 25 μg/ml and 50 μg/ml for imatinib and, after validation for the analysis of the main metabolite, with purified N-desmethyl-imatinib, respectively. The analysis was performed by UV detection at 260 nm.

This system had a detection limit of 10 ng/ml for imatinib and N-desmethyl-imatinib using 300 μl plasma, urine or CSF. Within-day variation was 7% for imatinib and 9% for N-desmethyl-imatinib at a concentration of 100 ng/ml. Daily variation determined on ten consecutive days with plasma spiked with 100 ng/ml of imatinib and N-desmethyl-imatinib was 4% and 6%, respectively. At the detection limit, the coefficient of variation was 11% for imatinib and 14% for N-desmethyl-imatinib as proven by ten measurements with spiked plasma samples. Similar results were found for the analysis in urine. For sample preparation, 30 μl concentrated perchloric acid was added to 300 μl plasma or RPMI and rapidly shaken in an autoshaker for 10 min. Afterwards, 200 μl of the enrichment eluent were added to the sample and the mixture was shaken for another 10 min. Proteins were then precipitated by centrifugation for 7 min at 4000 *g*. Of the resulting supernatant, 200 μl was injected into the HPLC system for analysis. CSF and urine were diluted with enrichment eluent (50/50, v/v) and after 7 min centrifugation at 4000 *g* a volume of 200 μl was injected without further preparation.

Pharmacokinetic calculations

The pharmacokinetic results were analyzed based on the TOPFIT computer program providing optimized variation coefficients between the observed and calculated data [15]. An optimal regression coefficient >0.94 was found in all patients using a linear two-compartment model assuming a classical first-order absorption constant described by the equation:

$$C_p = A'_1 e^{-at} + A'_2 e^{-\beta t} - A'_3 e^{-kat}$$

where Cp is the plasma concentration at a specific time point, t is time, A_1 to A_3 are dimensionless coefficients required to describe the time-course in a specified compartment, a and β are elimination rate constants, and ka is the absorption rate constant.

As a weighting function for the measured data $1/y^2$ was used. The plasma decay curve of N-desmethyl-imatinib was determined independently of the respective data of the parental compound, assuming a first-order rate constant of metabolism. Kinetic parameters as derived from this procedure revealed a close correlation with the results calculated by applying a linear user-defined compartment model in which the measured data of imatinib and N-desmethyl-imatinib were fitted together. Since the amounts of imatinib that had been absorbed and metabolized were unknown, dose-dependent data such as the distribution volume at pseudo-steady-state (V_{ss}) and total body clearance ($clearance_{total}$) were approximated by assuming a mean bioavailability of 100% for imatinib and a mean metabolized amount of 20% for N-desmethyl-

imatinib, as suggested by published and total urine elimination data.

Imatinib

Imatinib was kindly provided by Novartis Pharma (Basel, Switzerland). The compound was dissolved in sterile water, filtered and stored at −20°C at a concentration of 10 m*M*.

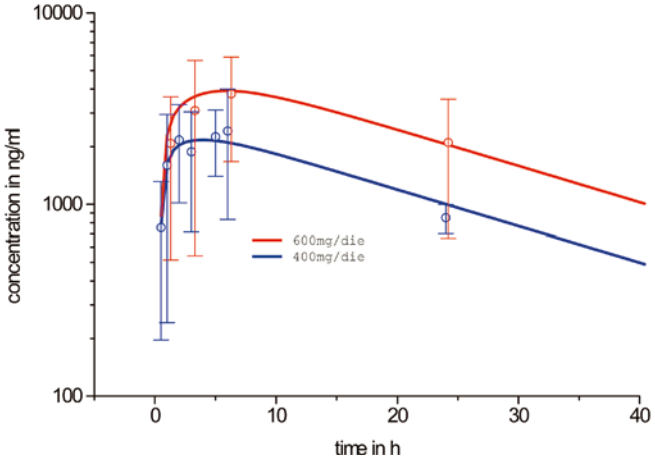


Fig. 1 The mean imatinib concentration-time curves over 24 h fitted by the model of all measured plasma concentrations to the data is shown. The *red curve* represents data from patients treated with 600 mg and the *blue curve* data from patients treated with 400 mg imatinib

Results

Peak plasma concentrations, AUC and terminal $t_{1/2}$ of imatinib

Mean plasma concentrations at the initiation of imatinib therapy were determined in 32 CML patients treated with either 400 or 600 mg imatinib. Figure 1 shows the mean plasma decay curves with standard deviations for the respective data points calculated from all measured data from patients 1 to 32 up to 40 h after imatinib administration at a dose of 400 or 600 mg orally per day. Table 2 shows all pharmacokinetic parameters calculated for individual patients as well as the mean values (patients 1 to 32). Peak concentrations of imatinib were reached 4 h after oral administration (range 4–5 μg/ml for the 600 mg dose and 2–3 μg/ml for the 400 mg dose). The average terminal half-life for both doses was 17 h. This indicates that steady-state concentrations would be reached 4–5 days after the first administration in a daily schedule.

The AUC showed an over-proportional level of 85.9 μg·h/ml for patients treated with 600 mg compared to 37.5 μg·h/ml for those treated with 400 mg. The discrepancy in these AUC values mainly resulted from patient no. 4 who showed an extreme AUC of 197 μg·h/ml due to a very high plasma concentration of 15.6 μg/ml. In this patient the half-life of imatinib remained in the normal range (17.8 h). Excluding this

Table 2 Pharmacokinetic parameters calculated from the individual plasma curves and mean of all data sets including the mean plasma curves calculated proportionately for 600 mg/day for patients 1–32

Patient no.	Dose (mg/day)	$t_{1/2terminal}$ (h)	$t_{1/2absorption}$ (h)	AUC (μg·h/ml)	C_{max} (μg/ml)	t_{max} (h)	V_{ss} (l)	$Clearance_{total}$ (ml/min)
1	600	10.2	0.46	63.8	5.03	2.24	128	157
2	600	15.4	4.27	34.7	2.06	7.82	108	288
3	600	17.2	1.34	75.6	5.19	4.13	78	132
4	600	17.8	1.52	197.0	15.6	3.69	46	51
5	600	13.2	1.58	74.1	7.70	3.19	102	112
6	600	27.3	0.31	70.0	4.98	1.96	336	143
Mean—patients[a]		16.9	1.58	85.9	6.76	3.84	133	147
CV (%)		35	90	66	69	55	78	53
Mean—all data points[b]		14.1	0.72	88.8	3.70	3.63	138	113
7	400	17.3	2.08	40.6	1.76	3.67	147	164
8	400	18.2	4.18	41.5	2.42	6.47	72	161
9	400	15.2	2.10	30.8	1.17	2.68	259	216
10	400	21.0	0.99	51.8	2.77	2.60	205	129
11	400	16.5	3.40	34.2	2.56	5.15	58	195
12	400	11.6	1.97	25.8	1.47	3.86	217	258
Mean—patients[a]		16.6	2.45	37.5	2.02	4.07	160	187
CV (%)		19	47	25	32	37	51	25
Mean—all data points[b]		18.2	1.73	39.5	1.91	3.30	220	169
All data sets								
Mean		16.7	1.9	71.5	4.80	3.89	151	163
CV		25.2	66.1	54.9	73.2	41.8	56.4	37.5
Median		16.9	1.43	63.0	4.33	3.65	133	159
Range		10.2–27.3	0.72–4.27	34.7–197.0	2.1–15.6	1.96–7.82	46–336	51–288

[a]Means of individual parameters calculated for every patient
[b]One calculation using the mean of all data points

Table 3 Pharmacokinetic data for patients 33–89 treated with 400 or 600 mg/day imatinib (values are means of the indicated patient groups) (*n.a.* not available, i.e. no sample)

	Plasma (ng/ml)	CSF (ng/ml)		Ratio (%)	24-h urine	
		No meningeosis	Meningeosis		Milligrams	Percent of dose
Imatinib	3372	40	32		n.a.	n.a.
CV (%)	43	56	67			
Patient nos.	33–89	73–86	87–89			
CSF/plasma imatinib				1.8		
CV (%)				71		
Patient nos.				73–89		
N-desmethyl-imatinib	579	< 10	< 10		n.a.	n.a.
CV (%)	84					
Patient nos.	33–89	73–86	87–89			
Imatinib	2785	n.a.	n.a.		33.2	5.6
CV (%)	34				33	
Patient nos.	90–94				90–94	
N-desmethyl-imatinib	483	n.a.	n.a.		13.4	2.2
CV (%)	54				67	
Patient nos.	90–94				90–94	
N-desmethyl-imatinib/imatinib						
Plasma				17		
CV (%)				74		
Patient nos.				33–94		
Urine				39		
CV (%)				47		
Patient nos.				90–94		

Table 4 Imatinib pharmacokinetics after discontinuation of imatinib prior to BMT conditioning therapy in two patients treated with 500 and 800 mg imatinib, respectively. Dose-dependent parameters were calculated for the first dose administered assuming 100% absorption of the imatinib dose

Patient no.	Dose (mg)	$t_{1/2}$ (h)		AUC (μg·h/ml)	C_{max} (μg/ml)	t_{max} (h)	V_{ss} (l)	Clearance (ml/min)		Imatinib in urine (% of dose)
		Terminal	Absorption					Total	Renal	
1	800	27.1	0.97	44.60	1.18	4.52	622	299	8.09	2.71
2	500	24.4	0.40	40.00	2.12	1.40	382	208	8.64	4.14

patient from calculation of the mean of the 600-mg group resulted in a nearly proportional AUC value compared to the 400-mg group (63.6 versus 37.5 μg·h/ml).

CSF analysis and renal elimination of imatinib

In the second group (patients 33–89) imatinib and N-desmethyl-imatinib in single point plasma samples were analyzed. Additionally, imatinib and N-desmethyl-imatinib levels were determined in CSF probes from 17 patients. Table 3 summarizes plasma and CSF concentrations using this approach. Three CSF samples was obtained from patients with meningeosis leukemica and 14 CSF samples were obtained from patients without meningeosis. All patients were treated with 400 mg or 600 mg per day imatinib and analyzed under steady-state conditions. Samples were taken between 3 and 8 h after imatinib administration. The imatinib plasma levels in this group are in the range of those found in the CML patients described above (Tables 2 and 3). More interestingly, the N-desmethyl-imatinib/imatinib ratio was 17%, but showed a distinct interindividual variability of 74%, probably reflecting different metabolic behavior between individual patients. The 24-h urine elimination showed a total elimination of 5.6% (33.2 mg) and 2.2% (13.4 mg) of the given dose for imatinib and N-desmethyl-imatinib, respectively.

The mean imatinib concentration of the CSF from all 17 patients analyzed was 38 ng/ml (39 and 34 ng/ml in CSF from patients without and with meningeosis, respectively) indicating that only a small fraction of plasmatic imatinib crosses the blood-brain barrier, regardless of the presence of meningeosis leukemica. N-Desmethyl-imatinib was not clearly detectable in CSF samples, but peak levels were below 10 ng/ml, reflecting a similar imatinib/N-desmethyl-imatinib ratio as found

Table 5 N-desmethyl-imatinib pharmacokinetics after discontinuation of imatinib prior to BMT conditioning therapy in two patients treated with 500 and 800 mg imatinib, respectively. Dose-dependent parameters were calculated for the first dose administered assuming 100% absorption and 20% metabolism of the imatinib dose

Patient no.	Dose (mg)	$t_{1/2}$ (h)		AUC (μg·h/ml	C_{max} (μg/ml)	t_{max} (h)	V_{ss} (l)	Clearance (ml/min)		N-desmethyl-imatinib in urine (% of dose)
		Terminal	Metabolism					Total	Renal	
1	800	94.5	0.39	12.50	0.90	3.11	1750	214	18.6	1.74
2	500	84.8	1.01	10.40	0.22	1.71	1100	160	16.0	2.00

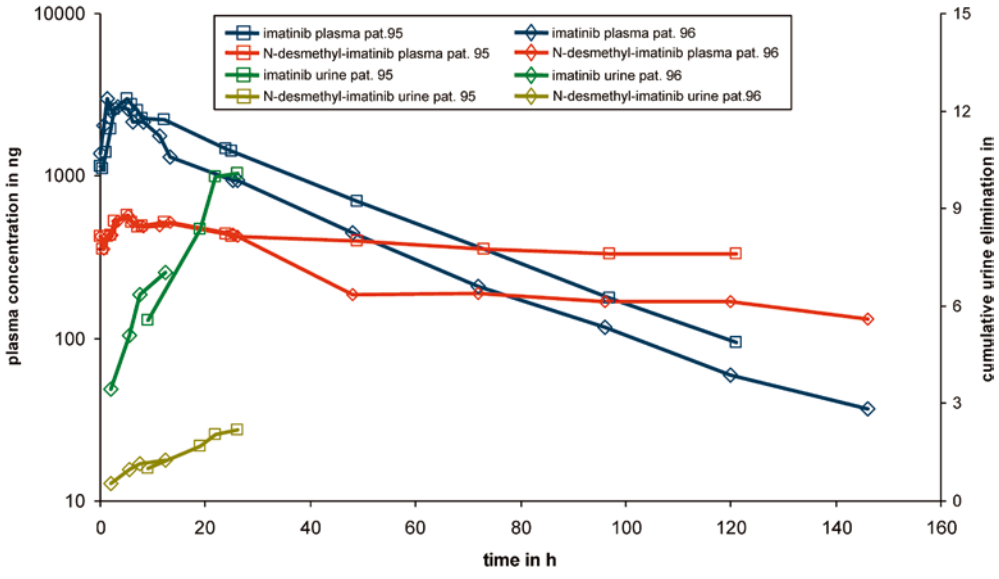


Fig. 2 The plasma and urinary concentrations of imatinib and N-desmethyl-imatinib in two patients. *Blue curves* (imatinib) and *red curves* (N-desmethyl imatinib) are Individual concentration-time curves derived following treatment of the patients with 500 mg or 800 mg imatinib. *Green* and *light-green* curves represent the corresponding cumulative urine elimination at steady-state (raw data)

in plasma samples. Since this level was under the evaluated detection limit of the HPLC method, we do not present the N-desmethyl-imatinib levels in CSF as a numerical result.

The concentrations of imatinib and N-desmethyl-imatinib in plasma and urine from two patients (nos. 95 and 96) after discontinuation of imatinib allowed the calculation of the pharmacokinetic parameters in Tables 4 and 5. In contrast to the patient group 1–32, we were able to draw samples over 7 days with declining imatinib and N-desmethyl-imatinib levels, allowing a thorough calculation of half-life for both substances (Fig. 2). Moreover, the renal clearance for both imatinib and N-desmethyl-imatinib was calculated based on the urinary elimination in these patients. The plasmatic parameters of these patients (Tables 4 and 5) were within the range of the data shown in Tables 2 and 3 ($t_{1/2}$ values for imatinib were 27.1 and 24.4 h, and for N-desmethyl-imatinib were 94.5 and 84.4 h, respectively). As expected, the renal clearances in these two patients were low: 8.09 and 8.64 ml/min and 18.6 and 16.0 ml/min for imatinib and N-desmethyl-imatinib, respectively.

Most importantly, the half-life of N-desmethyl-imatinib dramatically exceeded the half-life of imatinib by nearly fourfold. Assuming a critical level for bcr-abl inhibition of 100 ng/ml total imatinib concentration (the intracellular protein-bound fraction has not been determined to date [3]), the wash-out time for effective concentrations of imatinib is about 100 h, but >200 h for N-desmethyl-imatinib (Fig. 3).

Cellular uptake of imatinib and N-desmethyl-imatinib

In HL-60 cells cultured in RPMI supplemented with fetal calf serum there was a dose-dependent accumulation of imatinib at concentrations ranging from 200 ng/ml to 46 μg/ml following incubation for 5 h (Fig. 4). This analysis was based on an approximate mean cellular volume of 50 μl/2×10^7 cells for HL-60 cells, and showed cellular concentrations of imatinib between 3.5 μg/ml at the lowest incubation concentration (200 ng/ml) and 950 μg/ml at the highest incubation concentration of 42 μg/ml imatinib.

To clarify the influence of protein binding of imatinib, we simultaneously incubated HL-60 cells with similar concentrations of imatinib in human plasma (200 ng/ml to 42 μg/ml). This approach resulted in approximately fivefold lower intracellular imatinib concentrations (range 3.4–610 μg/ml), indicating that indeed only non-protein bound imatinib contributed to

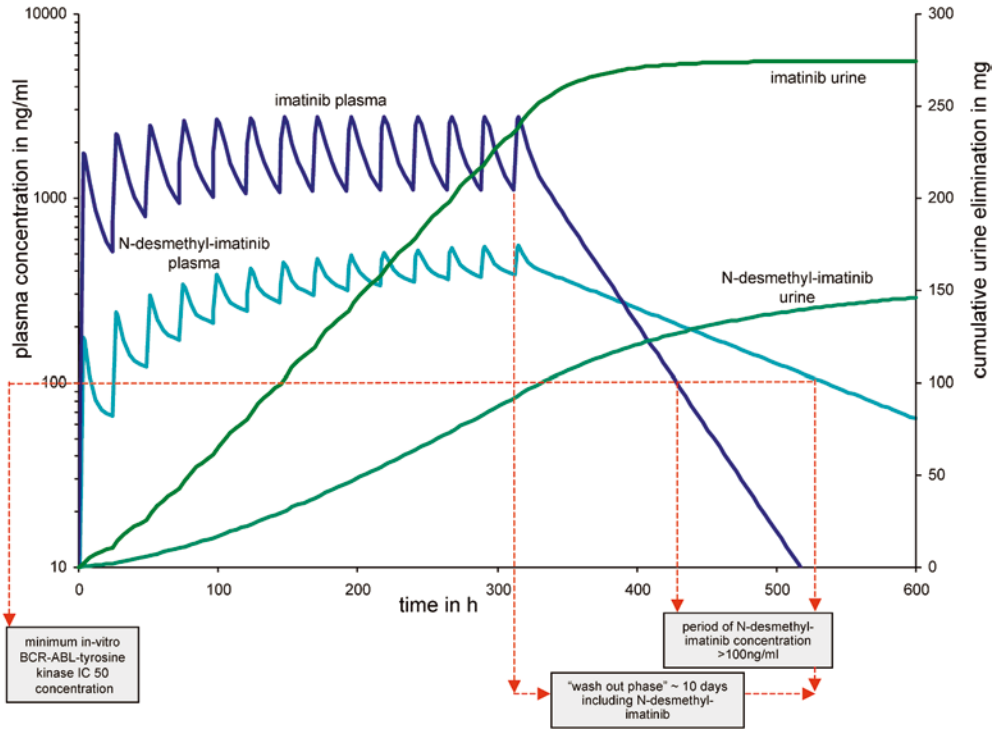


Fig. 3 Simulated concentration-time curve and cumulative urine elimination for imatinib and N-desmethyl-imatinib assuming a 14-day administration period (mean data from patients 95 and 96)

cellular uptake (Fig. 4). Similar observations were made for N-desmethyl-imatinib (Fig. 5). However, in these experiments only a limited amount of N-desmethyl-imatinib was available and extracellular incubation concentrations ranged from only 65 to 6600 ng/ml. At these low—but clinically important—extracellular concentrations, uptake of N-desmethyl-imatinib was in the range 1.25–124 μg/ml and seemed to be higher than that of imatinib. To strengthen this observation more experiments with more incubation concentrations of N-desmethyl-imatinib are required.

Discussion

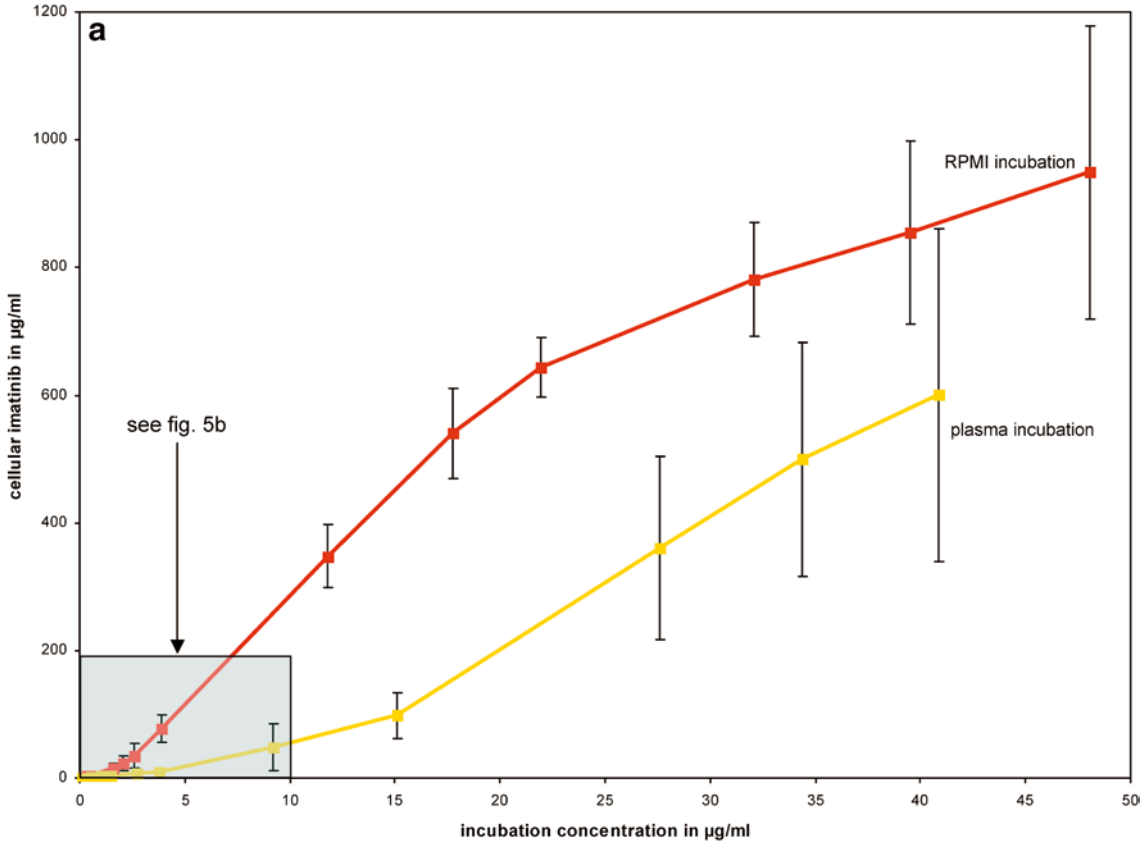
Despite the numerous reports of the clinical efficacy of imatinib and the biologic mechanisms involved, only a limited amount of data are presently available on its pharmacokinetic properties both in an in-vitro and in an in-vivo setting. Especially in view of the various mechanisms of resistance to imatinib that have been described so far [16, 17, 18], precise pharmacokinetic data in relation to clinical response are needed, as in a significant number of non-responders none of the underlying mechanisms of resistance can be identified.

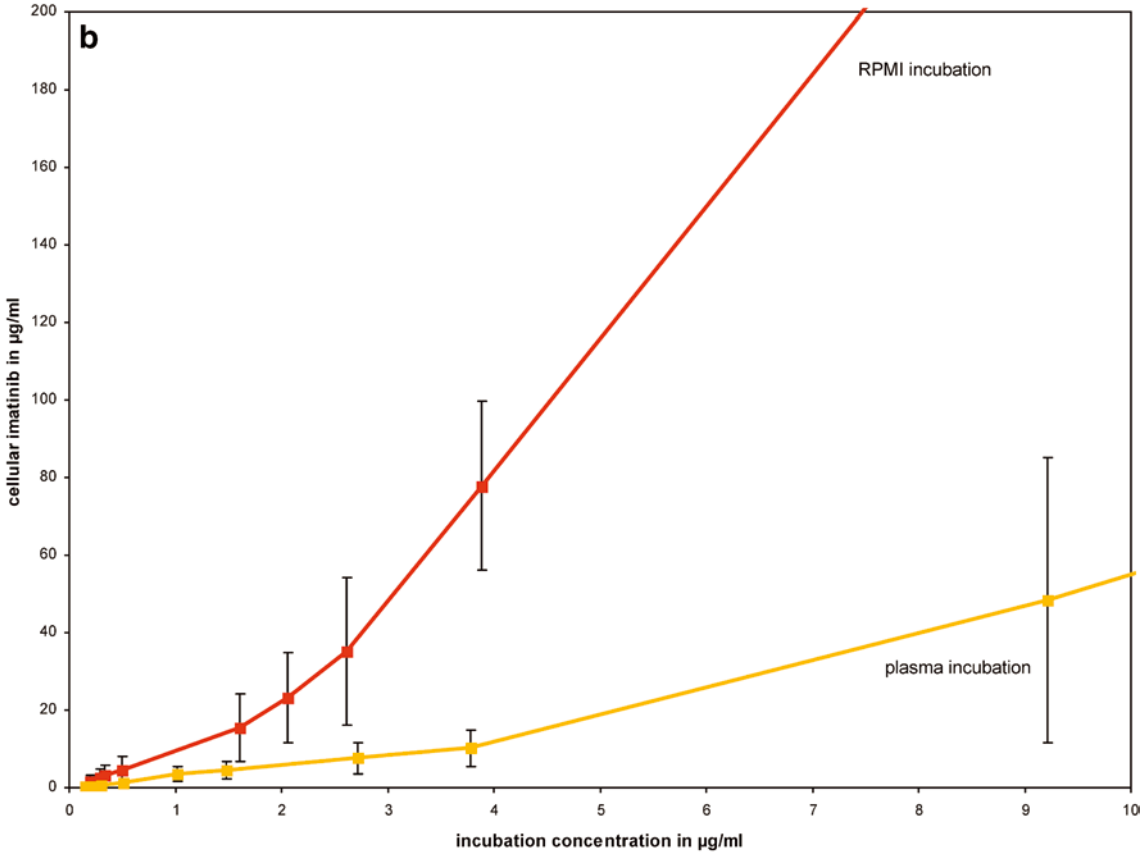
Bakhtiar et al. [19] used liquid chromatography-tandem mass spectrometry to quantify both imatinib and its main metabolite CGP74588 in human plasma. In this study, nominal concentrations of imatinib ranging from 4.0 to 10,000 ng/ml could be detected. However, only limited data on patient plasma are presented and pharmacokinetic parameters as well as inter- and intraindividual variations were not evaluated.

In our series of patients, peak plasma concentrations (C_{max}) of imatinib were detected after a mean time of 3.84 and 4.07 h following oral intake of 400 or 600 mg imatinib, respectively. These findings are comparable to those recently reported by Gambacorti et al. [12] who found a C_{max} after 1 and 3 h following oral intake of 400 mg imatinib. Likewise, the parameters mean $t_{1/2}$, AUC and C_{max} were comparable in our group of patients (16.6 h, 37.5 μg/ml·h and 2.02 μg/ml in the 400-mg group and 16.9 h, 85.9 μg/ml·h and 6.76 μg/ml in the 600-mg group) to those reported by Gambacorti et al. (12.5 h, 24.66 μg/ml·h and 2.352 μg/ml in the 400-mg group and 12.5 h, 99.74 μg/ml·h and 7.83 μg/ml in the 600-mg group). Patient no. 4 showed excessive AUC and C_{max} values but with a half-life of imatinib in the usual range. Clinically, this patient had a 6.5-year history of CML and was treated with 600 mg imatinib because of the presence of accelerated phase for 32 months with continuous hematologic response over 28 months. Interestingly, in this patient no signs of severe toxicity were observed, despite the relatively high plasma level of imatinib. The assumption of significant alterations caused by interindividual variability of imatinib is further supported by the observation that intraindividual values of AUC are relatively stable.

The calculated half-life of imatinib in this group of patients regardless of dose was 16.6 h (range 10.2–27.3 h), resulting in an approximate time to reach pseudosteady-state plasma levels of 96–120 h. Further, there was a linear correlation between dose of imatinib and the AUC.

Degradation of imatinib predominantly occurs hepatically in a CYP3A4-dependent pathway.


a
RPMI incubation
plasma incubation
see fig. 5b
cellular imatinib in µg/ml
incubation concentration in µg/ml
0
200
400
600
800
1000
1200
0
5
10
15
20
25
30
35
40
45
50


b
RPMI incubation
plasma incubation
cellular imatinib in µg/ml
incubation concentration in µg/ml
0
20
40
60
80
100
120
140
160
180
200
0
1
2
3
4
5
6
7
8
9
10

◀

Fig. 4 **a** Cellular imatinib uptake in 2×10^7 HL-60 cells after incubation with imatinib for 5 h. The data from three independent experiments over 3 months are shown. Each experiment was done in triplicate. **b** Shaded area of **a** shown enlarged (imatinib concentrations 0–10 μg/ml)

N-Desmethyl-imatinib is the main metabolite of a group of metabolites comprising piperazine-N-oxide-imatinib, hydroxymethyl-phenyl-imatinib and pyridine-N-oxide-imatinib (Fig. 6).

According to the manufacturer, N-desmethyl-imatinib has similar inhibitory effects on the tyrosine kinase activity of bcr/abl, c-kit, PDGF-R and c-abl to those of imatinib. However, the plasmatic ratios of imatinib and all other metabolites and their biologic activity have not yet been established. As the oxygen residues in piperazine-N-oxide-imatinib, hydroxymethyl-phenyl-imatinib and pyridine-N-oxide-imatinib will eventually prevent the binding of these molecules to the imatinib binding pocket and because of their relatively low urinary concentrations (data not shown) this work focused on the biologic activity of N-desmethyl-imatinib.

The mean N-desmethyl-imatinib plasma concentrations in our patients (531 ng/ml) under pseudosteady-state conditions, although only 17% of the imatinib plasma concentration, were above the nominal and effective concentrations of imatinib in an in vitro setting (100 ng/ml), indicating the clinical significance of this

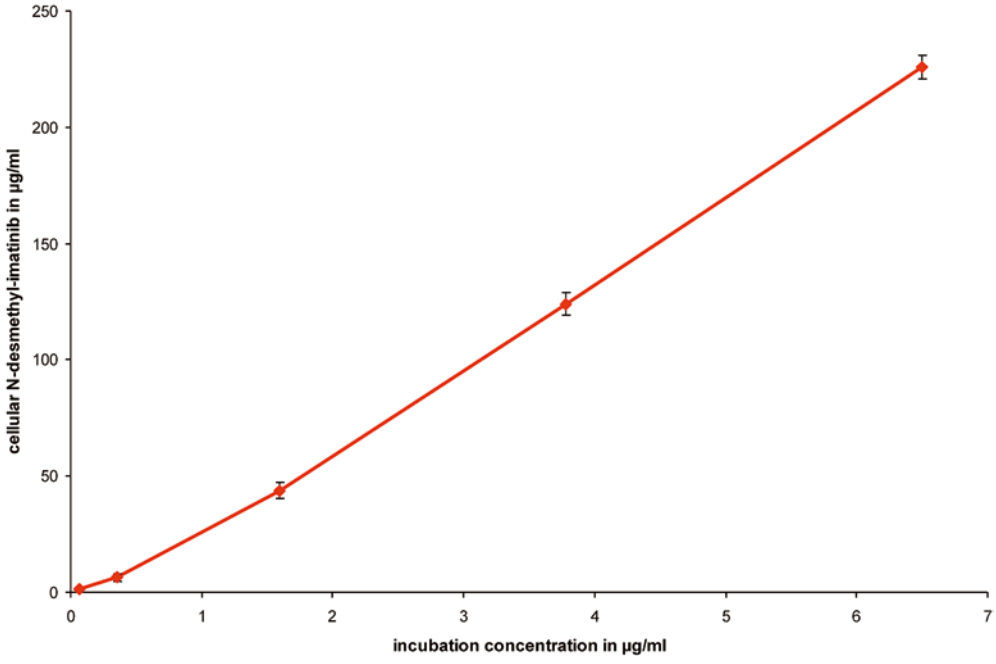


Fig. 5 Cellular N-desmethyl-imatinib uptake in 2×10^7 HL-60 cells after incubation with N-desmethyl-imatinib for 5 h in RPMI medium. The experiment was done in triplicate

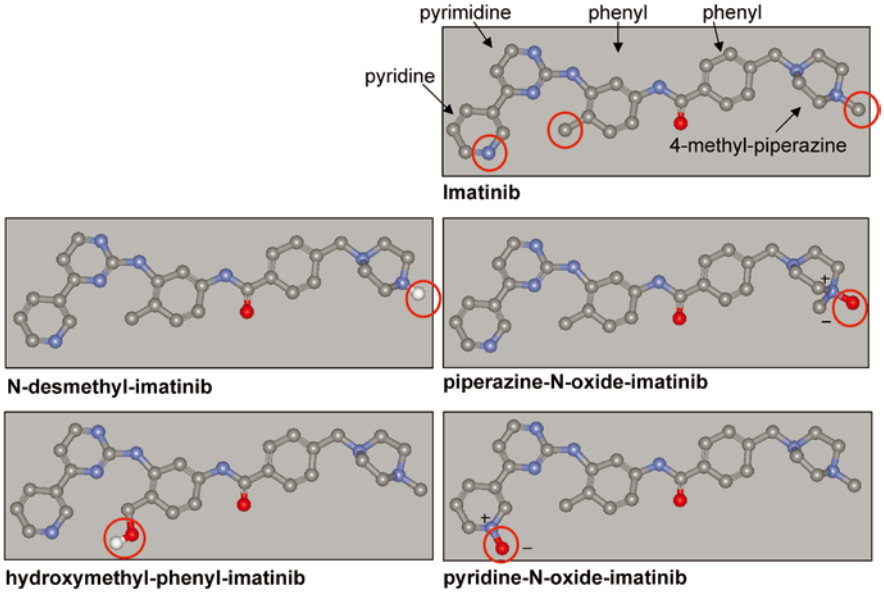


Fig. 6 Chemical structures of imatinib and its metabolites (altered groups in the metabolites are marked with *circles*)

substance. However, biologically significant competition between imatinib and its main metabolite N-desmethyl-imatinib for the imatinib-binding site still needs to be evaluated.

It is noteworthy that the $t_{1/2}$ of N-desmethyl-imatinib greatly exceeded the $t_{1/2}$ of imatinib. Discontinuation of imatinib in two patients resulted in a rapid decrease in plasma imatinib concentrations while plasma N-desmethyl-imatinib concentrations only moderately declined in the same period (> 140 h) (Fig. 2). Likewise, urinary levels of imatinib exceeded those of N-desmethyl-imatinib and were characterized by a more rapid decline (Fig. 2). Based on these results, we derived approximate plasma concentration-time curves and cumulative urinary elimination for imatinib and N-desmethyl-imatinib (Fig. 3). This calculation allowed us to estimate the wash-out phase of both imatinib and N-desmethyl-imatinib after discontinuation of treatment below a plasma concentration of 100 ng/ml. Figure 3 shows that despite relatively low concentrations of the metabolite under steady-state conditions, the wash-out phase of N-desmethyl-imatinib significantly exceeded the plasma elimination of imatinib (200 h versus 100 h). This observation may be of clinical significance, as it is still unclear whether continuation of imatinib treatment might increase the intensity of toxicity in myeloablative conditioning therapy in allogeneic stem cell transplantation.

Further, our results confirm a renal elimination of imatinib of 5–10% and a renal clearance of about 10 ml/min, indicating that imatinib pharmacokinetics will not be significantly affected in patients with compensated renal failure. Although there are no data on the issue of hemofiltration and imatinib levels, we assume that because of the high distribution volume and plasma binding, dialysis may not substantially interfere with imatinib plasma levels. Overall, based on these findings we can assume that the main elimination route of imatinib and its main metabolite is via the intestine.

In a subgroup of our patients we additionally compared imatinib levels in CSF samples obtained from ALL patients with or without meningeosis leukemica. As expected, intrathecal concentrations of imatinib were much lower in the CSF than in the plasma, indicating limited penetration of imatinib across the blood-brain barrier. In 14 patients with no signs of meningeal leukemia imatinib concentrations were approximately the same (38 ng/ml) as in three patients with proven meningeosis leukemica (34 ng/ml). Apparently, even in patients in whom the integrity of the blood-brain barrier is impaired by meningeosis leukemica, oral treatment with imatinib will not be a therapeutic option.

Although the involvement of the MDR1 gene (multiple drug resistance 1 gene) in the intracellular pharmacokinetics of imatinib is yet not fully understood, it is of note that p-glycoprotein overexpression has been shown to confer resistance to imatinib in a leukemia cell line model [20]. Further, Dai et al. [21] have demonstrated that intracellular flux of imatinib can be significantly altered by administration of the p-glycoprotein inhibitor LY335979 and that the brain-to-plasma concentration ratio of imatinib is elevated in mdr1a/b$^{-/-}$ knockout mice. These findings indicate a potential role for p-glycoprotein in the transfer of imatinib to the CSF. We therefore speculate whether the concomitant administration of verapamil, an effective inhibitor of p-glycoprotein, could become a therapeutic option in this group of patients.

As previous studies have demonstrated the emergence of intrinsic resistance in vitro (i.e. amplification of the Philadelphia chromosome) on exposure of cells to increasing concentrations of imatinib, the equilibrium of extra- and intracellular imatinib concentrations certainly plays a critical role in the emergence of resistant clones [16, 17].

The determination of intracellular imatinib concentrations in HL-60 cells demonstrated a dramatic influx of imatinib when compared to the extracellular concentrations as illustrated by the hyperbolic increase in cellular imatinib uptake from both human plasma and RPMI medium (Fig. 4). These results lead to the conclusion that apparently minor alterations in extracellular imatinib concentrations may result in a dramatic shift in the intracellular imatinib concentration. This observation was most significant at extracellular concentrations ranging between 2 and 20 μg/ml, indicating that an active transporter mechanism may be involved in the intracellular uptake of imatinib. However, we admit that clinically relevant concentrations are below this range. Nevertheless, a recent study has demonstrated that incubation of K562 cells with a variety of tyrosine kinase inhibitors including imatinib results in a dose-dependent reduction in intracellular [^{3}H]uridine uptake, indicating that tyrosine kinase inhibitors may eventually affect nucleoside transport [22].

Additionally, our results add to the observation that in vivo resistance to imatinib in mice may be mediated by increased protein binding through α1-acid glycoprotein [10, 11, 12, 13]. RPMI medium has a lower protein concentration than human plasma and therefore will bind imatinib less effectively. We therefore speculate that the higher cellular influx of imatinib into HL-60 cells incubated in RPMI was a consequence of a relatively low protein content in the medium.

Finally, we also provide data on the cellular uptake of N-desmethyl-imatinib (Fig. 5). Here, we observed an overproportional intracellular increase similar to the cellular uptake of imatinib and a tendency to higher intracellular concentrations, i.e. better uptake compared to imatinib.

In view of the relatively wide range of peak plasma concentrations observed in individual patients, we finally assume that the oral dosages of imatinib administered to patients do not necessarily guarantee effective concentrations in the target cell. Assuming that plasma imatinib concentrations do reflect the intracellular concentration, therapeutic imatinib levels should always

exceed a threshold range of 0.1 to 5 μM. Therefore, thorough determination of imatinib levels could be considered essential in patients lacking adequate hematologic and cytogenetic responses.

Acknowledgement P. le Coutre, K.-A. Kreuzer, O. Ottmann and G. Ehninger are principal investigators/coinvestigators of clinical trials with imatinib carried out by Novartis Pharmaceuticals.

References

1. Buchdunger E, Zimmermann J, Mett H, et al (1996) Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by 2 phenylaminopyrimidine derivative. Cancer Res 56:100–104
2. Druker BJ, Tamura S, Buchdunger E, et al (1996) Effects of a selective inhibitor of the Abl tyrosinekinase on the growth of BCR-ABL positive cells. Nat Med 2:561–566
3. Gambacorti-Passerini C, le Coutre P, Mologni L (1997) Inhibition of the ABL kinase activity selectively blocks the proliferation of BCR-ABL$^+$ leukemic cells and induces apoptosis. Blood Cells Mol Dis 23:380–394
4. Deininger MW, Goldmann JM, Lydon N, Melo J (1997) The tyrosine kinase inhibitor CGP57148B selectively inhibits the growth of BCR-ABL-positive cells. Blood 90:3691–3698
5. le Coutre P, Mologni L, Cleris L, et al (1999) In vivo eradication of human BCR-ABL-positive leukemia cells with an ABL kinase inhibitor. J Natl Cancer Inst 91:163–168
6. Kantarjian H, Sawyers C, Hochhaus A, et al (2002) Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. N Engl J Med 346:645–652
7. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, Cornelissen JJ, Fischer T, Hochhaus A, Hughes T, Lechner K, Nielsen JL, Rousselot P, Reiffers J, Saglio G, Shepherd J, Simonsson B, Gratwohl A, Goldman JM, Kantarjian H, Taylor K, Verhoef G, Bolton AE, Capdeville R, Druker BJ (2003) IRIS Investigators. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 348:994–1004
8. Druker BJ, Sawyers CL, Kantarjian H (2001) Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 344:1038–1042
9. Talpaz M, Silver RT, Druker BJ, Goldman JM, Gambacorti-Passerini C, Guilhot F, Schiffer CA, Fischer T, Deininger MW, Lennard AL, Hochhaus A, Ottmann OG, Gratwohl A, Baccarani M, Stone R, Tura S, Mahon FX, Fernandes-Reese S, Gathmann I, Capdeville R, Kantarjian HM, Sawyers CL (2002) Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. Blood 99:1928–1937
10. Gambacorti-Passerini C, Barni R, le Coutre P, et al (2000) Role of α1 acid glycoprotein in the in vivo resistance of human BCR-ABL+ leukemic cells to the Abl inhibitor STI571. J Natl Cancer Inst 92:1641–1650
11. le Coutre P, Kreuzer KA, Na IK, et al (2002) Determination of α-1 acid glycoprotein in patients with Ph+ chronic myeloid leukemia during the first 13 weeks of therapy with STI571. Blood Cells Mol Dis 28:75–85
12. Gambacorti-Passerini C, Zucchetti M, Russo D, Frapolli R, Verga M, Bungaro S, Tornaghi L, Rossi F, Pioltelli P, Pogliani E, Alberti D, Corneo G, D'Incalci M (2003) Alpha1 acid glycoprotein binds to imatinib (STI571) and substantially alters its pharmacokinetics in chronic myeloid leukemia patients. Clin Cancer Res 9:625–632
13. Gambacorti-Passerini CB, Rossi F, Verga M, Ruchatz H, Gunby R, Frapolli R, Zucchetti M, Scapozza L, Bungaro S, Tornaghi L, Rossi F, Pioltelli P, Pogliani E, D'Incalci M, Corneo G (2002) Differences between in vivo and in vitro sensitivity to imatinib of Bcr/Abl+ cells obtained from leukemic patients. Blood Cells Mol Dis 28:361–372
14. Ford JM (2002) Imatinib mesilate, investigators' brochure, 5th edn. Novartis Pharma
15. Heinzel G, Hammer R, Wolf M, Koss FW, Bozler G (1977) Model building in pharmacokinetics/Part III: simplified rules for the deduction of analytical solutions for linear compartment models (in German). Arzneimittelforschung 27:904
16. le Coutre P, Tassi E, Varella-Garcia M, et al (2000) Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells trough gene amplification. Blood 95:1758–1766
17. Weisberg E, Griffin JD (2000) Mechanism of resistance to the ABL tyrosine kinase inhibitor STI571 in BCR/ABL-transformed hematopoietic cell lines. Blood 95:3498–3505
18. Gorre ME, Mohammed M, Ellwood K, et al (2001) Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 293:876–880
19. Bakhtiar R, Lohne J, Ramos L, Khemani M, Hayes M, Tse F (2002) High-throughput quantification of the anti-leukemia drug STI571 (Gleevec^{TM}) and its main metabolite (CGP 74588) in human plasma using liquid chromatography-tandem mass spectrometry. J Chromatogr B 768:325–340
20. Mahon FX, Belloc F, Lagarde V, Chollet C, Moreau-Gaudry F, Reiffers J, Goldman JM, Melo JV (2003) MDR1 gene overexpression confers resistance to imatinib mesylate in leukemia cell line models. Blood 101:2368–2373
21. Dai H, Marbach P, Lemaire M, Hayes M, Elmquist WF (2003) Distribution of STI-571 to the brain is limited by p-glycoprotein-mediated efflux. Pharmacol Exp Ther 304:1085–1092
22. Huang M, Wang Y, Cogut SB, Mitchell BS, Graves LM (2003) Inhibition of nucleoside transport by protein kinase inhibitors. J Pharmacol Exp Ther 304:753–760