In vitro blood distribution and plasma protein binding of the tyrosine kinase inhibitor imatinib and its active metabolite, CGP74588, in rat, mouse, dog, monkey, healthy humans and patients with acute lymphatic leukaemia

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Aims

To determine blood binding parameters of imatinib and its metabolite CGP74588 in humans and non-human species.

Methods

The blood distribution and protein binding of imatinib and CGP74588 were determined *in vitro* using ¹⁴C labelled compounds.

Results

The mean fraction of imatinib in plasma (f_p) was 45% in dog, 50% in mouse, 65% in rat, 70% in healthy humans and up to 92% in acute lymphatic leukaemia (AML) patients. Similarly, f_p for CGP74588 was low in dog and monkey (30%), higher in rat, mouse and humans (70%) and highest in some AML patients (90%). The unbound fraction of imatinib and CGP74588 in plasma was lower in rat, mouse, healthy humans and AML patients (2.3–6.5% at concentrations ≤ 5000 ng ml⁻¹) compared to monkey and dog (7.6–19%). Both compounds displayed high binding to human α_1 -acid glycoprotein. AML patients had a reduced haematocrit and showed greatest variability in their blood binding parameters.

Conclusion

Imatinib and CGP74588 displayed very similar blood binding parameters within all species/groups investigated. The five species clustered into two distinct groups with rat, mouse and humans being clearly different from dog and monkey. For both compounds, higher protein binding was associated with a decreased partitioning into blood cells.

Introduction

Glivec[®] (imatinib mesylate, Novartis Pharma, Basel, Switzerland; Gleevec[™] in the US) is a rationally designed, potent and highly selective tyrosine kinase inhibitor [1, 2], with a favourable toxicity profile. It is

marketed for the treatment of chronic myeloid leukaemia (CML) and gastrointestinal stromal tumours (GISTs).

Resistance to Glivec[®] has occurred in some patients with leukaemia [3] and GIST [4]. Different mecha-

nisms of resistance, including increased plasma protein binding, have been suggested [5–9]. CGP74588 – the primary metabolite of imatinib – is pharmacologically active [10], and shows a similar potency and selectivity profile as the parent drug (Novartis, unpublished results). Here we report the blood distribution and plasma protein binding of imatinib and CGP74588 in species used in preclinical safety assessment as well as in healthy humans and in patients with acute myeloid leukaemia (AML), undergoing currently clinical trial evaluation.

Methods

Materials

[¹⁴C]imatinib (3.3–3.4 MBq/mg, 98% pure) and [¹⁴C]CGP74588 (2.7–3.3 MBq/mg, 98% pure) were synthesized in the Novartis Pharma Isotope Laboratory. Centrifree devices YM30 were obtained from Amicon (Beverly, MA, USA), Soerensen buffer from Gibco (Paisley, Scotland), and human serum albumin (HSA, A1887) and α_1 -acid glycoprotein (AGP, G8995) from Sigma (St. Louis, USA). Blood was taken from healthy males ($n = 3$, 37–59 years) and from female AML patients ($n = 5$, 67–81 years). Patients gave written informed consent before taking part in the study, which was approved by the ethics committee of the Landesärztekammer Rheinland-Pfalz, Mainz (Germany). Blood was taken from male albino rats (Hanover, Wistar), from male p53 wild type mice (B6.129-Trp53^{tm1Brd+} N5), from male beagles dogs and from one labrador dog. Blood was also obtained from cynomolgous monkeys (Centre de Primatologie, Niederhausbergen, France). Heparin was used as the anticoagulant.

Blood distribution

Fresh blood was spiked to give the desired concentrations of imatinib and CGP74588 (see Table 1), and incubated for 30 or 60 min at 37 °C. Cells were sedimented by centrifugation (800–1500 g, 10–20 min, 37 °C). Concentration was measured in blood (C_b) before and in plasma (C_p) after centrifugation, and haematocrit (H) was determined, all in triplicate. The blood distribution of imatinib was not determined in the monkey. The fraction in plasma (f_p), the concentration in blood cells (C_{bc}) and the blood cell to plasma concentration ratio ($K_{bc/p}$) were calculated as follows:

$$\begin{aligned} f_p(\%) &= (C_p/C_b) \times (1 - H) \times 100 \\ C_{bc} &= [C_b - C_p \times (1 - H)]/H \\ K_{bc/p} &= C_{bc}/C_p \end{aligned}$$

Plasma protein binding

Control experiments in plasma water and Soerensen buffer indicated that ultrafiltration is a suitable method: free-permeation >0.75, recovery >85%. Plasma (platelet-free) incubations were for 10, 30 or 60 min at 37 °C before centrifugation in Centrifree devices (10 min, 2000 g, 37 °C). Radioactivity was determined in plasma (C_i) and the ultrafiltrate (C_u). The unbound fraction (f_u) was calculated as follows:

$$f_u(\%) = C_u/C_i \times 100$$

HSA and AGP were dissolved in Soerensen buffer pH 7.4 at concentrations of 40 and 1 mg ml⁻¹, respectively. After spiking with drug, the samples were incubated (30 min, 37 °C) before ultracentrifugation (200000 g, 5 h, 37 °C) of 1 ml aliquots. Radioactivity was measured in the samples before (C_i) and in the supernatant after centrifugation (C_u). Recoveries of drug and metabolite were about 95% and there was negligible auto-sedimentation in buffer.

Statistical analysis

All given p -values are based on 2-sided t -tests unless otherwise stated. As a measure of dispersion the standard deviation (SD) is used. When comparing the difference between means, the 95% confidence interval (CI) of the difference is given. Fixed-effects models with 'group' or 'compound' as categorical variable and concentration/haematocrit as covariates were fitted to the f_u and f_p data and least-squares means were statistically compared.

Results

Patients, from whom samples were taken before imatinib treatment, showed greater variability in blood binding parameters compared to healthy subjects, and could be divided into two distinct subgroups (AML I, $n = 3$ and AML II, $n = 2$) (Table 1 + 2). In those AML subgroups, fractions in plasma (f_p) and unbound fractions in plasma (f_u) differed significantly. The differences between the 2 subgroups (based on fixed effects models) with respect to f_p were 15.8% (95% CI of the difference: 12.5–19%, $p < 0.001$) and 15.3% (95% CI of the difference: 12.7–17.9%, $p < 0.001$) for imatinib and CGP74588, respectively. With respect to f_u , the differences between the 2 subgroups were 1.8% (95% CI of the difference: 1.1–2.4%, $p < 0.001$) and 1.4% (95% CI of the difference: 0.8–2.0%, $p < 0.001$) for imatinib and CGP74588, respectively. Finally, with respect to haematocrit values, the difference between the 2 subgroups was 0.07 (95% CI of the difference: 0.05–0.09, $p = 0.001$). Only for the purpose of analysing the effect of plasma protein binding on blood distribution the patients were treated as two groups.

Table 1Blood distribution of [^{14}C]imatinib and [^{14}C]CGP74588

Blood Concentration [ng/mL]	Healthy humans H = (0.44 – 0.47)	Fraction of compound in plasma [%]		AML Patients I + II H = (0.29 – 0.37)
		AML Patients I H = (0.36 – 0.37)	AML Patients II H = (0.29 – 0.30)	
[14C]Imatinib				
300–500	74 (3)	75 (2)	92 (2)	82 (9)
5000	67 (4)	73 (3)	87 (2)	79 (8)
10000	69 (2)	NA	NA	NA
25000	60 (2)	NA	NA	NA
[14C]CGP74588				
100	68 (3)	71 (1)	87 (2)	78 (9)
300	71 (1)	75 (3)	90 (0.4)	81 (9)
500	72 (4)	75 (3)	90 (1)	81 (8)
	Rat H = 0.44	Mouse H = 0.46	Dog H = 0.48*	Monkey H = 0.52
[14C]Imatinib				
<100	63 (3)	NA	NA	NA
100–1000	NA	51 (0.5)	47 (1)	NA
5000–10000	68 (NA)	50 (1)	45 (NA)	NA
20000	65 (NA)	NA	42 (NA)	NA
	Rat (H = 0.52)	Mouse (H = 0.46)	Dog (H = 0.49)	Monkey (H = 0.52)
[14C]CGP74588				
50–100	77 (0.4)	66 (1)	32 (1)	30 (1)
500	74 (1)	67 (0.3)	32 (1)	31 (1)
5000	66 (1)	66 (1)	33 (1)	31 (0.1)

Data are presented as mean (standard deviation). H: hematocrit; NA, not available; *Fp calculated based on average in-house historical H.

Blood distribution was species dependent, with similar patterns for both compounds (Table 1). For CGP74588 the group means of f_p in species with extensive partitioning into blood cells (dog and monkey; mean and standard deviation (SD): $31 \pm 1\%$) differed significantly (95% CI difference: 28.7%–50.4%, $p = 0.001$) from that in the group with limited partitioning into blood cells (healthy humans, rat, mouse; group mean and s.d. $71 \pm 5\%$). For imatinib, no monkey data were measured; again f_p values in humans, rat and mouse were higher than in dog.

All AML patients had a strongly reduced haematocrit, as expected from their disease state. Against this background, the blood cell to plasma concentration ratios were determined to assess differences in blood distribution. Amongst humans the means of these ratios were found to be significantly different between the three groups ($p < 0.01$ for all 6 comparisons, no individual

statistics given) for both compounds, however, they were not different when comparing all AML patients and healthy volunteers ($P > 0.8$, no individual statistics given) for both compounds: AML I (0.57 ± 0.05 , 0.64 ± 0.09) > healthy volunteers (0.41 ± 0.07 , 0.50 ± 0.07) > AML II (0.21 ± 0.06 , 0.27 ± 0.08), all AML (0.43 ± 0.19 , 0.49 ± 0.20); given numbers are means and SDs for imatinib (300–500 ng mL $^{-1}$), and CGP74588 (100–500 ng mL $^{-1}$), respectively.

Plasma protein binding of [^{14}C]imatinib and its N-desmethyl metabolite [^{14}C]CGP74588 was species dependent (Table 2). For both compounds, high binding was found in humans and the two rodent species with f_u values of 2.3–6.5%. For humans, f_u increased to about 11% at very high [^{14}C]imatinib concentrations. In the limited number of patient samples analysed, no strongly reduced f_u was found. In monkey and dog plasma protein binding was lower for both compounds. Overall, a

Table 2Plasma protein binding of [14 C]imatinib and [14 C]CGP74588

	Healthy humans	Unbound fraction of compound in plasma [%]		
		AML Patients I	AML Patients II	AML Patients I + II
[14C]Imatinib				
300–500	4.3 (0.2)	5.6 (0.6)	3.7 (0.2)	4.8 (1.1)
5000	5.8 (1.0)	6.5 (0.4)	5.0 (1.0)	5.9 (1.0)
12000	8.3 (NA)	NA	NA	NA
26000	11.2 (NA)	NA	NA	NA
[14C]CGP74588				
100	3.1 (0.7)	4.3 (0.6)	3.4 (0.5)	3.9 (0.7)
300	4.4 (0.6)	5.1 (0.3)	3.6 (0.1)	4.5 (0.8)
500	4.8 (0.6)	5.3 (0.1)	3.7 (0.4)	4.6 (0.9)
	Rat	Mouse	Dog	Monkey
[14C]Imatinib				
100–1000	NA	2.4 (0.4)	NA	10.1 (NA)
90–16000	5.5 (0.2)	NA	19 (0.4)	NA
[14C]CGP74588				
50–100	2.5 (0.0)	2.5 (0.1)	11.9 (0.3)	7.6 (0.3)
500	3.2 (0.1)	2.3 (0.1)	13.4 (0.2)	8.5 (0.1)
5000	3.5 (0.1)	2.4 (0.2)	13.9 (0.0)	9.2 (0.1)

Data are presented as mean (standard deviation); NA, not available.

minor increase of f_u with increasing compound concentration was observed. Group means and SDs for f_u in the species with higher binding (healthy humans, rat, mouse) and the species with lower binding (dog, monkey) were 4.1 ± 1.6 vs. 14.6 ± 6.3 for imatinib (300–500 ng ml $^{-1}$ for humans) and 3.2 ± 0.9 vs. 10.8 ± 3.3 for CGP74588 (all reported concentrations), respectively. The differences between the 2 groups were 10.5% (95% CI of the difference: -0.7 – 21.7% , $p = 0.06$) and 7.6% (95% CI of the difference: 1.6 – 13.5% , $p = 0.03$) for imatinib and CGP74588, respectively.

In humans, when comparing the two compounds, imatinib and CGP74588, no significant differences in blood binding (f_u and f_p) parameters were observed. The differences between the 2 compounds (using a fixed effects model with concentration and haematocrit as covariates) amounted to 1.7% for f_p (95% CI of the difference: -1.7 – 5.1% , $p = 0.32$) and to 0.02% for f_u (95% CI of the difference: -0.61 – 0.65% , $p = 0.94$).

Both CGP74588 and imatinib (300–500 ng ml $^{-1}$) were highly bound to isolated human AGP (f_u 1.7 and 3.1% at 1 g l $^{-1}$, respectively) and moderately bound to HSA ($f_u \sim 20\%$ for both compounds at 40 g l $^{-1}$).

Discussion

Differences in plasma protein binding of a drug between species or individuals have an impact on pharmacokinetics and can influence the pharmacological activity [12, 13]. For imatinib elevated levels of AGP have been related to *in vivo* resistance in mouse [9] and humans [14, 15], whereas other investigators did not observe any relevant binding of imatinib to AGP employing fluorescence quenching [16]. The data presented here, based on direct measurement of drug concentrations with radiolabelled compounds, confirmed high binding to AGP. Clinical data have demonstrated a correlation between AGP levels and plasma imatinib concentrations [17]. However, for imatinib as a moderately to low clearance drug with a high volume of distribution [11], a drastic effect of changes in protein binding on the pharmacological response is unlikely [18]. A recent study confirmed this: administration of Clindamycin, an AGP bound compound, caused a fourfold increase in the f_u of imatinib, with an almost unchanged unbound concentration [17]. Variability in plasma protein binding can thus contribute to the variability in exposure (based on total plasma concentration) observed in clinical trials [11], but will not affect the unbound and hence pharmacolog-

ically active drug concentration for a low hepatically cleared drug such as imatinib.

Plasma protein binding of CGP74588 the major metabolite of imatinib is important, because of its pharmacological activity. Differences in the rate of metabolism between individuals resulting in different imatinib/CGP74588 ratios, could cause differences in pharmacological activity, if plasma protein binding of the drug versus the metabolite was different. Our data demonstrate that CGP74588 displays very similar characteristics as imatinib: high binding to AGP and a similar pattern among species.

Comparing protein binding and blood distribution among different species indicated that protein binding appeared to be a driving force for the blood distribution of CGP74588 and imatinib. Higher binding to plasma proteins (in humans, rat and mouse) reduced partitioning into blood cells, compared to species with lower protein binding (dog and monkey). This relation was also found for humans, when AML patients were considered as two groups. The f_u values between the three human groups were in accordance with the ranking of blood cell to plasma concentration ratios (AML I > healthy volunteers > AML II) showing the link between plasma protein binding and blood cell uptake. Since only a small number of patient blood samples was analysed in this study, the determined parameters might not be representative for the whole AML patient population.

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