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SHORT COMMUNICATION

Prospective Analysis in GIST Patients on the Role of Alpha-1 Acid Glycoprotein in Imatinib Exposure

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Abstract

Background For imatinib, a relationship between systemic exposure and clinical outcome has been suggested. Importantly, imatinib concentrations are not stable and decrease over time, for which several mechanisms have been suggested. In this study, we investigated if a decrease in alpha-1 acid glycoprotein (AGP) is the main cause of the lowering in imatinib exposure over time.

Methods We prospectively measured imatinib trough concentration (C_{min}) values in 28 patients with gastrointestinal stromal tumours, at 1, 3 and 12 months after the start of imatinib treatment. At the same time points, AGP levels were measured.

Results Overall, imatinib C_{min} and AGP levels were correlated ($r^2 = 0.656$; $P < 0.001$). However, AGP levels did not fluctuate significantly over time, nor did the change in AGP levels correlate with the change in the imatinib C_{min}.

Conclusion We showed that systemic AGP levels are not likely to be a key player in the decrease in systemic imatinib exposure over time. As long as intra-individual changes in imatinib exposure remain unexplained, researchers should standardize the sampling times for imatinib in order to be able to assess the clinical applicability of therapeutic drug monitoring.

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Key Points

The decrease in systemic imatinib exposure during the first months of treatment is not likely to be caused by increased clearance due to fluctuations in alpha-1 acid glycoprotein levels.

Since systemic imatinib exposure varies over time, pharmacokinetic sampling should be performed at standardized time points.

1 Background

Imatinib is one of the first tyrosine kinase inhibitors (TKIs) for which therapeutic drug monitoring (TDM) is deemed suitable in the treatment of both chronic myeloid leukaemia (CML) and gastrointestinal stromal tumours (GISTs). In CML, higher imatinib exposure has been found in patients with a treatment response [1–4], and imatinib trough concentration (C_{min}) values above 1000 ng/mL have been found to be predictive of higher response rates [1, 2]. For GIST, the target imatinib C_{min} has been established in a phase II study, in which patients with an imatinib C_{min} in the lowest quartile (i.e. below 1100 ng/mL) had significantly worse progression-free survival (PFS), and it was suggested that this concentration should serve as a target C_{min} [5]. Studies conducted in the context of routine care have shown that more than half of imatinib-treated patients do not reach that C_{min} [6–8]. In these studies performed in daily practice, the C_{min} was measured more than 3 months after the start of treatment, whereas in the study describing the threshold of 1100 ng/mL, the imatinib C_{min} was

established after 4 weeks of treatment. Meanwhile, it has been shown that imatinib clearance increases—and systemic concentrations therefore decrease—during the first 3 months of treatment [9, 10]. Hence, it could be expected upfront that an even larger proportion of patients than the 25 % in the phase II study would have a C_{min} below 1100 ng/mL when it was determined later than 3 months after the start of treatment, and doubts have been raised as to whether this threshold set at a time when systemic exposure has not yet stabilized is indeed the appropriate target imatinib C_{min} in patients with GIST [11]. Accordingly, in one of the more recent retrospective studies in GIST patients, a threshold of 760 ng/mL led to better prediction of the outcome [8]. In the same study population, however, the median PFS was longer for patients with a C_{min} >1100 ng/mL than for those with a C_{min} >760 ng/mL (67 versus 56 months) [8].

Several mechanisms have been suggested to account for the reduction in systemic imatinib concentrations over time, the first being decreased absorption [9]. Alternatively, Chatelut et al. [12] proposed that systemic imatinib exposure decreases because of increased clearance rather than because of decreased absorption. As imatinib is predominantly bound to the acute-phase protein alpha-1 acid glycoprotein (AGP) [13–15], a reduction in AGP over time would lead to less protein-bound imatinib and therefore a larger proportion of free imatinib that could be metabolized or excreted [12]. According to this theory, it is assumed that a decrease in the tumour burden leads to a reduced inflammatory syndrome, which in turn causes lowering of AGP levels. The finding that changes in AGP levels over time correlate well with changes in imatinib concentrations seems to back this mechanism [16]. However, these data were analysed in retrospect and, more importantly, they were not collected in a structured manner, as imatinib concentrations and AGP levels were measured at separate time points. Additionally, AGP levels and imatinib concentrations were not assessed synchronously in that study. To firmly establish the influence of AGP levels on blood imatinib concentrations, this study aimed to prospectively assess the correlation between imatinib C_{min} values and AGP levels.

2 Methods

2.1 Patients

Adult patients with GIST in whom commencement of imatinib treatment was planned were eligible for inclusion in this study. The exclusion criteria were prior imatinib treatment within 3 months prior to the start of the study,

major surgery within 2 weeks prior to the start of the study, use of potent cytochrome P450 (CYP) 3A inhibitors or inducers, and inability to give or understand informed consent. The study protocol was approved by the local institutional review board (protocol number MEC13-203). All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all individual participants included in the study.

2.2 Study Procedures

Pharmacokinetic sampling was performed at 1, 3 and 12 months after the start of imatinib treatment. At each time point, two blood samples were collected in addition to the standard-of-care blood draw at scheduled outpatient visits. The first sample was collected in a glass tube containing lithium heparin as an anticoagulant, and was used to quantify the concentrations of imatinib and its main metabolite, CGP74588. This sample was processed to plasma within 30 minutes by centrifugation for 15 min at $2500 \times g$ (4 °C). Next, the plasma was transferred to polypropylene tubes and stored at −70 °C until the time of analysis at the Laboratory of Translational Pharmacology, Erasmus MC Cancer Institute (Rotterdam, the Netherlands). The methods used for quantification of imatinib and CGP74588 concentrations have been described previously [17]. The second sample was collected in a serum-separating tube and processed to serum. Serum AGP levels were measured using an immunoturbidimetric assay on a Cobas Integra 800 (Roche Diagnostics GmbH, Mannheim, Germany) in accordance with the instructions of the manufacturer. Briefly, serum AGP was agglutinated with a polyclonal goat-antihuman AGP antibody. The amount of agglutination of the antigen–antibody complex was measured turbidimetrically.

2.3 Statistical Considerations

At least 24 patients had to be included to identify a rho value of 0.55 in a two-sided test with alpha = 0.05 and power = 0.8. Correlation was tested using Pearson's correlation, equality of two means was tested using t tests and equality of more than two means was tested using one-way analysis of variance (ANOVA). P values <0.05 were considered statistically significant. Descriptive statistics were performed using IBM SPSS Statistics version 21 software (SPSS, Chicago, IL, USA). All other statistical analyses were performed using GraphPad Prism 5.0 software (GraphPad Software, La Jolla, CA, USA).

3 Results

3.1 Baseline

Between April 2013 and March 2015, 35 patients signed informed consent and were included in the study. Four patients were not evaluable because they stopped imatinib treatment within 3 months and therefore did not provide repetitive pharmacokinetic samples. In another three patients, only one C_{min} value was available because the patients had taken imatinib prior to the other sampling time points or because they had an imatinib concentration below the limit of quantification (<20.0 ng/mL). The baseline characteristics of the 28 evaluable patients are depicted in Table 1. Eight of the evaluable patients stopped treatment before the final sampling time point because of progressive disease (n = 3), cessation of neoadjuvant treatment (n = 4) or toxicity (n = 1).

Table 1 Baseline patient characteristics

Characteristic	Patients, $n = 28$
Age at start	
Years	69 (10)
Sex	
Male	16 (57 %)
Female	12 (43 %)
WHO performance status	
0	12 (43 %)
1	13 (46 %)
2	1 (4 %)
Unknown	2 (7 %)
c-KIT mutation	
Wild type	5 (18 %)
Exon 9	6 (21 %)
Exon 11	12 (43 %)
Exon 13	3 (11 %)
Unknown	2 (7 %)
Treatment setting	
Neoadjuvant	11 (39 %)
Adjuvant	5 (18 %)
Palliative	12 (43 %)
Dose at start	
300 mg QD	1 (4 %)
400 mg QD	26 (93 %)
800 mg QD	1 (4 %)

All values are presented as n (%) or as mean (standard deviation)

c-KIT KIT proto-oncogene receptor tyrosine kinase, QD once daily, WHO World Health Organization

3.2 AGP Levels and Imatinib Concentrations

A total of 73 imatinib trough concentration samples were obtained. In 69 cases, a blood sample for measurement of the AGP level was collected synchronically. The mean values of AGP, imatinib and CGP74588 at each time point are depicted in Table 2. At any of the three time points, the AGP levels in the five patients treated in the adjuvant setting did not differ significantly from those in the patients treated in the neoadjuvant or palliative settings (Fig. 1a).

3.3 AGP Versus Imatinib

Overall, AGP levels were significantly correlated with imatinib concentrations ($r^2 = 0.656$; $P < 0.001$; Fig. 2) and with the sum of imatinib and CGP74588 concentrations ($r^2 = 0.667$; $P < 0.001$). The correlation between imatinib concentrations and AGP levels was less strong in the 25 samples that were taken at the first time point after 30 days ($r^2 = 0.526$; $P < 0.001$; Fig. 3) in comparison with the correlations assessed at the two later time points. The absolute difference in AGP levels between time points 1 and 2 was also significantly correlated with the absolute difference in imatinib concentrations between time points 1 and 2 ($r^2 = 0.381$; $P = 0.002$) and between time points 1 and 3 ($r^2 = 0.355$; $P = 0.03$). The relative differences in AGP levels and imatinib concentrations between time points were not significantly correlated. The geometric mean AGP levels did not differ significantly between the three time points ($P = 0.141$; Fig. 1b).

4 Discussion

In this prospective setting, imatinib pharmacokinetics were closely correlated with systemic AGP levels when all samples obtained at the three different time points were considered together ($r^2 = 0.656$; $P < 0.001$). Although—at first sight—this appeared to be in line with the hypothesis that the increase in imatinib clearance is due to reduced systemic AGP levels [14, 16], the differences in AGP levels and imatinib C_{min} values between the time points were less strongly correlated. Moreover, the argument that AGP decreases during treatment and thereby contributes to increased imatinib clearance over time [12] did not seem to hold true, as we did not find substantial reductions in AGP levels during treatment ($P = 0.141$; Fig. 1). Patients treated in the adjuvant setting even had a gradual increase in AGP levels, which contradicted the theory that AGP levels are initially elevated because of an inflammatory syndrome directly after tumour surgery and decline over time when the surgery effects resolve [12]. Even though the decrease in imatinib concentrations was

Table 2 Analyses of the samples obtained at the different time points

	Time point 1, $n = 25$	Time point 2, $n = 25$	Time point 3, $n = 19$	Total, $n = 69$
Actual time since start of imatinib [days][a]	30 (3)	97 (30)	364 (20)	
AGP level [g/L][b]	0.97 (0.85–1.10)	0.81 (0.69–0.94)	0.89 (0.78–1.00)	0.89 (0.82–0.96)
Imatinib concentration [ng/mL][b]	1457 (1155–1838)	1305 (1001–1702)	1193 (967–1472)	1325 (1158–1516)
CGP74588 concentration [ng/mL][b]	308 (247–384)	265 (205–343)	231 (179–299)	270 (235–309)
Imatinib + CGP74588 concentration [ng/mL][b]	1777 (1420–2224)	1578 (1217–2047)	1439 (1165–1777)	1606 (1407–1833)
Correlation between imatinib and AGP[c]	0.526 ($P < 0.001$)	0.839 ($P < 0.001$)	0.411 ($P = 0.003$)	0.656 ($P < 0.001$)

The three time points are the times at which sampling was scheduled according to the protocol, i.e. 30 days, 90 days and 365 days after the start of treatment

AGP alpha-1 acid glycoprotein

[a] Units of time are presented as mean (standard deviation)

[b] Values are presented as geometric mean (95 % confidence interval)

[c] Correlations are depicted as r^2 (*P* value)
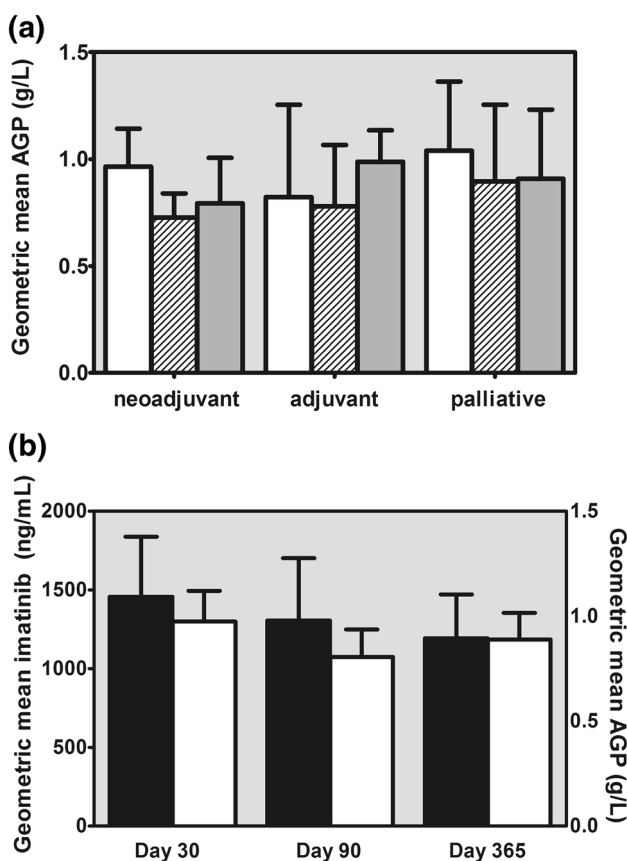

(a)

(b)

Fig. 1 **a** Geometric mean levels of alpha-1 acid glycoprotein (AGP) at 30 days (*white bars*), 90 days (*striped bars*) and 365 days (*dark grey bars*) from the start of treatment in each treatment setting. **b** Geometric mean trough concentrations of imatinib (*black bars*) and AGP (*white bars*) at each time point. The *error bars* represent the 95 % confidence intervals

Fig. 2 Correlation between imatinib concentrations and alpha-1 acid glycoprotein (AGP) levels in all samples ($n = 69$)

Fig. 3 Correlation between imatinib concentrations and alpha-1 acid glycoprotein (AGP) levels at 30 days ($n = 25$)

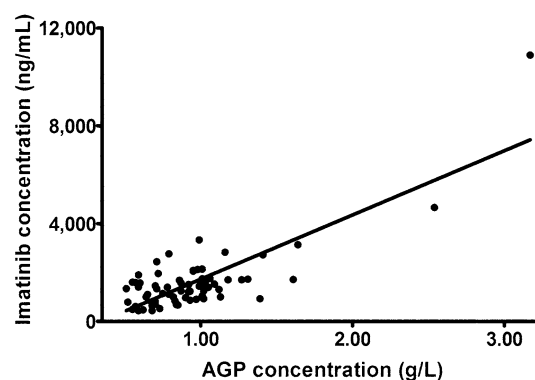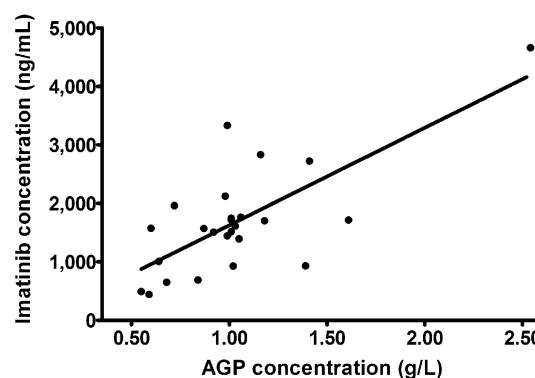not as large as those published previously, the implication of our current findings is that the role of systemic AGP levels in the reduced systemic imatinib exposure over time is relatively small and that other factors, e.g. reduced bioavailability, likely have larger influences on systemic exposure. Still, AGP might seriously interfere with imatinib exposure in vivo, as extravascular AGP affects imatinib pharmacokinetics beyond the systemic circulation [15], and preclinical research has shown that the

pharmacodynamic effects of imatinib are reduced in the presence of AGP [18–20]. Nonetheless, it remains questionable whether these extravascular effects can be used to determine the optimal dose for individual patients.

Unfortunately, the available evidence for individualized imatinib dosing in GIST patients is currently not robust, hampering assessment of the clinical relevance of TDM in GIST. Imatinib C_{min} values measured at different time points during treatment have previously been related to the clinical outcome [5, 8]. Also, as mentioned previously, because of the decrease in systemic imatinib concentrations over time, target C_{min} values after 1 month cannot be extrapolated into a dosing algorithm for the entire treatment period. Although it has been proposed that TDM be performed only after imatinib pharmacokinetics have stabilized after 3 months of treatment [11], whether or not an individual with GIST receives the proper treatment and dose would ideally become visible much earlier during treatment. For example, by using fludeoxyglucose (18F) [^{18}F-FDG] positron emission tomography [PET] as early as a few days after the start of treatment, it is possible to know whether or not a GIST patient is responding to treatment [21]. Either way, TDM in imatinib treatment can reach its full potential only when sampling times are standardized between research groups [22]. The sampling schedule employed in the current study could serve as a blueprint for larger studies because it incorporated C_{min} values at 1 month and at later time points, enabling assessment of long-term pharmacokinetic targets, which could subsequently be compared with the established target at 1 month. Alternatively, long-term pharmacokinetic targets could be derived from the target C_{min} at 1 month, using a formula that corrects for the parameters that contribute to the decrease in imatinib exposure. However, as the biological mechanism of this decreased exposure seems to be complex and multifactorial, the latter option to determine long-term pharmacokinetic targets is not likely to be computed soon. In parallel, other challenges in making TDM clinically usable will be to integrate the dosing range (300–800 mg daily) and the possible options in the case of insufficient concentrations (dose escalation or a treatment switch), but these are secondary to standardization of the sampling time points. Last, but certainly not least, it remains to be proven that imatinib TDM in GIST really translates into a better outcome in terms of either less toxicity or better anti-tumour effects.

5 Conclusion

We found that systemic AGP levels are not likely to be a key player in the decrease of systemic imatinib exposure over time. We believe that TDM is a very potent tool to improve personalized imatinib treatment, but it can flourish only if researchers ensure that their results are obtained in a standardized way.

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