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Everolimus alters imatinib blood partition in favour of the erythrocyte

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Abstract

The signal transduction inhibitor imatinib is one of the latest breakthroughs in cancer pharmacotherapy. It is administered orally over prolonged periods of time for the treatment of gastrointestinal stromal tumours. Routine therapeutic drug monitoring of blood plasma versus red blood cells over several years by liquid chromatography coupled tandem mass spectrometry has highlighted a very intriguing phenomenon. Imatinib plasma availability decreases dramatically owing to a significant shift in the partition ratio of red blood cells versus plasma. The shift is enforced by combination with everolimus, another signal transduction inhibitor. These data warrant routine erythrocyte versus plasma monitoring to prevent unexpected alterations in drug efficacy during long-term treatment.

Introduction

Erythrocytes (red blood cells; RBC) are involved in the transport and delivery of oxygen. Recently, it has become clear that erythrocytes can also fulfil an important role in the transport and delivery of substances other than oxygen, including pharmacotherapeutic agents (Dumez et al 2004b). Mathematical concepts of substance binding to erythrocytes were developed at the end of the last century (Hinderling 1997). The monitoring of pharmacotherapeutic agents in blood is generally limited to the plasma fraction. This is mainly due to technical difficulties that must be overcome when monitoring the cellular fraction of the blood, and the widely held view that plasma analysis alone generates sufficient information. It is estimated that less than 1% of clinical pharmacokinetic studies of anticancer agents deal with the monitoring of a specific cellular compartment (Dumez et al 2004a). However, it has been demonstrated over the past 15 years that monitoring pharmacologically active agents in the cellular fractions of blood, especially the RBC compartment, can yield important information concerning in-vivo drug behaviour (Wildiers et al 2002). The RBC compartment is of special interest owing to its overwhelming harvesting capacity; it constitutes by far the largest surface and volume theoretically available. With the advent of the measurement of sediment (MESED) device for quantitative isolation of substances associated with particles in suspension, interesting data have been generated regarding the presence of anticancer agents (particularly cyclophosphamide and melphalan) in erythrocytes (Highley et al 1996; Wildiers et al 2002).

Combinations of pharmacologically active agents may well influence partition between RBCs and plasma (partition ratio). This has been described for gemcitabine and taxotere (Dumez et al 2005). Furthermore, gender and smoking have been shown to influence the partition ratio of anticancer agents, although the generally accepted hypothesis that RBCs are loaded as a function of the free fraction, which in turn is dependent on total blood concentration, is still valid. Here we describe surprising alterations in the erythrocyte harvesting of imatinib, with in excess of 80% of the circulating drug binding to these cells.

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Materials and Methods

Collection of blood samples

We have used MESED technology in clinical trials of the signal transduction inhibitor imatinib (Gleevec; Novartis Pharma AG), and imatinib in combination with everolimus, over the past 5 years (van Oosterom et al 2001, 2005; Verweij et al 2003). Before patient registration, written informed consent was obtained according to national/local regulations. The protocol was approved by the local ethical committee. Imatinib was analysed in the erythrocytes and plasma of gastrointestinal stromal tumour (GIST) patients at regular time points using liquid chromatography coupled tandem mass spectrometry (LC-MS/MS) as described previously (Guetens et al 2003). Briefly, approximately 0.5 mL of blood was processed according to the MESED procedure as previously described for cyclophosphamide and melphalan (Highley et al 1996; Wildiers et al 2002). The first centrifugation at 3000 *g* generated plasma for further analysis. RBCs were quantitatively isolated (100 μL) at a precise ratio of 95.7:4.3 (RBC/plasma). The plasma is needed for the maintenance of the equilibrium that is lost when washing procedures are used (Driessen et al 1994). RBCs were lysed using HPLC water and then centrifuged. The supernatant was then processed for LC-MS/MS analysis in a similar fashion to plasma.

LC-MS/MS

An isocratic LC-MS/MS method for the specific and selective quantitation of imatinib in both RBCs and plasma has been used (Guetens et al 2003). The method includes a single protein precipitation step by the addition of acetonitrile to both the RBC isolate and plasma. The sample mixture was centrifuged at 3000 *g* for 10 min and the supernatant filtered through a HPLC filter (0.45 μm). The analytes of interest, imatinib and the internal standard (d8 deuterated imatinib), were eluted on a Waters Symmetry C(18) column (50 × 2.1 mm inner diameter, 3.5-μm particle size) using a 0.05% methanol/ammonium acetate (72:28, v/v) mixture. Imatinib and the internal standard were detected by electrospray MS/MS in the positive mode, and monitored in the multiple reaction monitoring transitions 494 > 394 and 502 > 394, respectively. The lower limit of quantification of imatinib was 2.1 ng mL^{-1} in RBCs and 1.8 ng mL^{-1} in plasma. The recovery from both plasma and RBCs was between 65% and 70%. The method proved to be robust, allowing simultaneous quantification of imatinib in RBCs and plasma with sufficient precision, accuracy and sensitivity. It is useful in monitoring the fate of the signal transduction inhibitor in various biological matrices, including RBCs.

Statistical analysis

Results are expressed as mean ± s.d. Statistical analysis of data was performed by using an unpaired Student *t*-test ($P < 0.05$ was indicative of a statistically significant difference). To determine the linear correlation between total blood concentration and the partition ratio, a linear regression model was used (calculated with Origin software).

Results and Discussion

Imatinib is a small molecule tyrosine kinase inhibitor of Bcr-Abl, KIT and platelet-derived growth factor receptor-α and -β. It is regarded as a breakthrough in the treatment of several malignancies, such as chronic myeloid leukaemia and GIST.

To determine the maximum partition ratio of imatinib, we have performed in-vitro loading experiments, using whole blood from human volunteers as a control. The partition ratio, that is the erythrocyte concentration versus plasma concentration, in the total blood of human volunteers, increases with the total blood concentration up to 0.28. This is in close agreement with pre-clinical data provided by the company that manufactures imatinib (Novartis).

Over the past 5 years we analysed imatinib in the erythrocytes and plasma of GIST patients at regular time points. Patient characteristics are shown in Table 1. The partition ratios of imatinib in patients treated for about 1 year with imatinib are within the range found during in-vitro loading experiments (Figure 1, Group 1). However, when imatinib is administered for longer periods of time, the partition ratio increases markedly above 0.46, even to values greater than 1, implying that the surplus of the circulating dose of the drug is present at the site of the erythrocyte (Figure 1, Group 2). In view of this increase, dose escalation might be required for efficacy (Verweij et al 2004). The mechanism responsible for this increase in partition ratio is not yet completely clear. It has been generally accepted that RBCs are loaded through the free fraction (Hinderling 1997). An increase or decrease in total plasma concentration would not necessarily result in a change of the free fraction concentration and/or RBC concentration. This is especially so since the free fraction is dependent on the presence of binding molecules, the characteristics of which may change during continuous treatment with imatinib.

Table 1 Patient characteristics

	Group 1	Group 2	Group 3
Number of patients	12	9	17
Glivec treatment (months)	9–15	26–34	18–41
Average Glivec treatment (months)	11.75	33	29
Number of Glivec doses			
400 mg	3	0	0
600 mg	3	5	17
800 mg	6	4	0
Number of blood samples	35	40	311
Mean partition ratio ± s.d.	0.17 ± 0.05	0.46 ± 0.28	0.70 ± 0.50

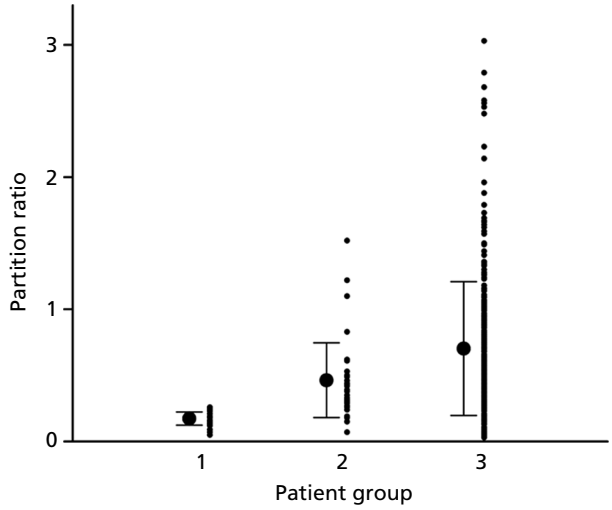

Figure 1 Mean partition ratio with standard deviation in patients treated with the signal transduction inhibitor imatinib for about 1 year (Group 1), after more than 2 years (Group 2) and in combination with another signal transduction inhibitor (Group 3).

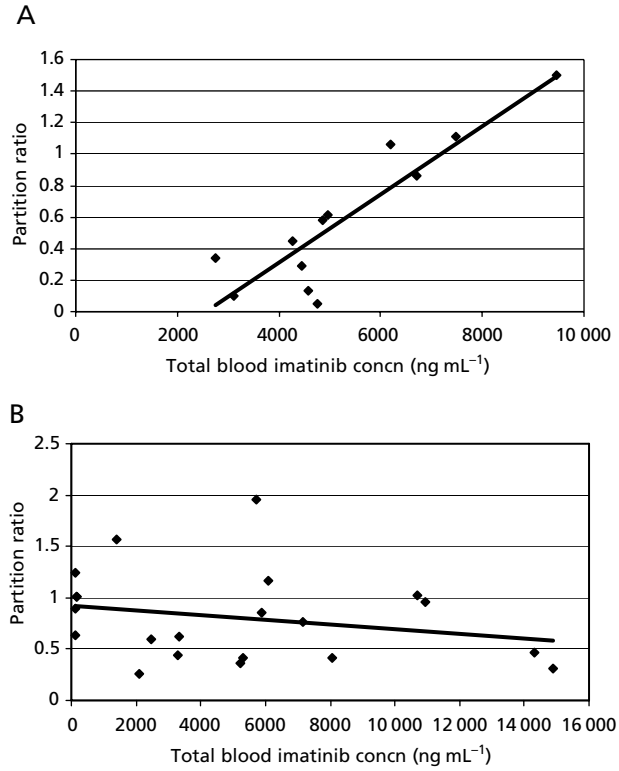

Figure 2 Linear relationship between total blood concentration of imatinib and partition ratio in the presence of everolimus in one patient ($P = 0.0001$) (A), and the loss of this relationship in another patient ($P = 0.81$) (B).

An important continuing problem when treating GIST patients with imatinib is the emergence of imatinib resistance. Combination with other tyrosine kinase inhibitors has been suggested as a way to overcome this resistance. Everolimus (RAD001; Novartis Pharma), an oral derivative of rapamycin, inhibits mTOR, a protein kinase downstream of AKT, involved in the regulation of cell growth, proliferation and survival. We further explored imatinib concentrations in erythrocytes versus plasma in GIST patients included in the combination study of imatinib and everolimus. In the majority of patients, a combination treatment with everolimus dramatically increases the partition ratio of imatinib, with values far above 0.31, the maximum value noted in the in-vitro experiments, with an almost negligible amount recovered from the plasma (Figure 1, Group 3). This effectively results in a marked dose reduction when strong binding to the erythrocyte hinders delivery. Indeed, inducing release by the addition of blank plasma to patient erythrocytes to reduce the partition ratio is less successful than with human volunteer erythrocytes (data not shown). Assuming that a sufficiently high imatinib plasma concentration is essential for a therapeutic effect, the loss of plasma availability may imply a dramatic loss of activity. The nature of the accumulation in RBCs is currently unknown and is the subject of further investigation. The reduced imatinib availability could explain the limited success of the combination treatment (van Oosterom et al 2005).

Another interesting finding is the dramatic change in the relationship between total blood concentration and partition ratio. Three of the patients treated with the imatinib/everolimus combination showed a partition ratio that was total blood concentration dependent (Figure 2A); this was not the case in the 14 other patients (Figure 2B).

Although it was stated almost a decade ago that there are many opportunities for challenging research in the underdeveloped area of RBC partitioning (Hinderling 1997), data on interactions between the partitioning profiles of concomitantly administered drugs remain extremely limited (Dumez et al 2004a). When gemcitabine is combined with taxotere, gemcitabine partition is altered (Dumez et al 2005). This could not be explained by data available on the partition of both drugs in octanol water systems (log P) or RBC partition data acquired for the drugs when present as a single agent. Moreover, this interaction can be dependent on gender and total blood concentration. With the combination of imatinib and everolimus, different mechanisms can be involved. (i) Competition at the level of plasma substances such as albumin, which are prone to bind the drugs of interest. When everolimus is bound to proteins normally available for imatinib, the imatinib free fraction will increase, and so will the RBC concentration, since RBC loading is generally accepted to be free fraction dependent (Hinderling 1997). (ii) The physiology of the RBC itself could change. Liphophilicity is generally seen as the main determinant for RBC uptake. The log P value of rapamycin and analogues (i.e. approx. 6) indeed is suggestive for a high partition ratio of RBC over plasma (Yatscoff et al 1993). It is known that everolimus is 75–95% bound to erythrocytes (Kovarik et al 2001; Armstrong & Streit 2003). Thus, the sequestering of everolimus may be sufficiently high to induce changes in RBC physiology, causing alterations in

the uptake capacity of imatinib. An extended in-vitro study is required to unravel this mechanism with a sufficient number of volunteers (≥10 per gender). (iii) Long-term exposure to one or both agents may well produce a physiologically altered RBC population by affecting erythropoiesis. This also requires extensive additional studies. A study of single agent everolimus and imatinib versus the combination in an octanol water system may give an early insight regarding possible influences on a shift of log P of both agents. However, it will not be indicative of the consequences for the uptake of the drugs into the RBC, as the target itself may change during exposure; the equations used for partition studies will no longer be valid since they are based on the assumption that binding constants are stable.

Conclusion

Marked changes in the partition ratio during long-term treatment with imatinib, or when imatinib is given in combination with everolimus, indicate an important, thus far unknown, role for the erythrocyte. The present data, together with other data concerning several anticancer agents, warrants routine monitoring of erythrocytes, as well as plasma, to prevent unexpected alterations in in-vivo drug behaviour. This is especially pertinent for anticancer agents, since they can affect the bone marrow, the site of origin of erythrocytes, and are therefore capable of inducing alterations in their own partition ratio.

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