Brief report

Imatinib mesylate (STI571) is a substrate for the breast cancer resistance protein (BCRP)/ABCG2 drug pump

Herman Burger, Hans van Tol, Antonius W. M. Boersma, Mariëlle Brok, Erik A. C. Wiemer, Gerrit Stoter, and Kees Nooter

Imatinib mesylate (STI571), a potent tyrosine kinase inhibitor, is successfully used in the treatment of chronic myelogenous leukemia and gastrointestinal stromal tumors. However, the intended chronic oral administration of imatinib may lead to development of cellular resistance and subsequent treatment failure. Indeed, several molecular mechanisms leading to imatinib resistance have already been reported, including overexpression of the MDR1/ABCB1 drug pump. We examined whether imatinib is a substrate for the breast cancer resistance protein (BCRP)/ABCG2 drug pump that is frequently overexpressed in human tumors. Using a panel of well-defined BCRP-overexpressing cell lines, we provide the first evidence that imatinib is a substrate for BCRP, that it competes with mitoxantrone for drug export, and that BCRP-mediated efflux can be reversed by the fumitremorgin C analog Ko-143. Since BCRP is highly expressed in the gastrointestinal tract, BCRP might not only play a role in cellular resistance of tumor cells but also influence the gastrointestinal absorption of imatinib. (Blood. 2004;104:2940-2942)

Study design

Reagents and cell lines

Imatinib and [14C]STI571 mesylate were obtained from Novartis (Basel, Switzerland). Ko-134 was used as a specific BCRP inhibitor.17 Mitoxantrone and doxorubicin were obtained from Pharmachemie (Haarlem, The Netherlands). MCF7 (ATCC, HTB-22); MCF7/MR, a mitoxantrone-resistant and BCRP-overexpressing subline; MCF7/AdVp3000 overexpressing the R482T BCRP variant; HEK293 cells transfected with pcDNA3 (HEK293/Neo); wild-type BCRP/ABCG2-R482R (HEK293/R); BCRP/ABCG2-R482G (HEK293/G); and BCRP/ABCG2-R482T (HEK293/T) were used.

Real-time RT-PCR

Relative mRNA expression for BCRP, MRP1, MRP2, and MDR1 was determined by real-time reverse transcriptase–polymerase chain reaction (RT-PCR) using an ABI PRISM 7700 (Applied Biosystems, Foster City, CA) and the comparative cycle threshold (Ct) method as described.18

Analysis of protein expression

BCRP protein levels were determined by Western blot analysis using BCRP-specific monoclonal antibody BXP-21 (1:200) as described previously.19

From the Department of Medical Oncology, Erasmus Medical Center Rotterdam, Daniel den Hoed Kliniek/Josephine Nefkens Institute, Rotterdam, The Netherlands.


Reprints: Herman Burger, Department of Medical Oncology, Erasmus MC/Josephine Nefkens Institute (Rm Be420), PO Box 1738, 3000 DR Rotterdam, The Netherlands; e-mail: h.burger@erasmusmc.nl.

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Accumulation of intracellular [14C]STI571 mesylate

Cells (10^6/mL) were exposed to [14C]STI571 (range, 0.1 μCi [3.7 kBq]-0.001 μCi [0.037 kBq]; 2.14 μM-0.021 μM imatinib) for 2 hours at 37°C and then extensively washed with ice-cold phosphate-buffered saline (PBS). Imatinib accumulation was calculated from the radioactivity measured in the cell pellet over that retained in the supernatant as determined by liquid scintillation counting. Relative accumulation (mean ± standard deviation [SD]), based on at least 3 independent experiments, is expressed as a fraction of that found in parental cells (% control).

Flow cytometry

Mitoxantrone and doxorubicin accumulation (fluorescence intensity) was determined by flow cytometry as described previously.20

Results and discussion

To determine whether imatinib is a substrate for the BCRP efflux pump, we examined 14C-labeled imatinib accumulation in MCF7/MR and MCF7/AdVp3000, 2 cell lines known to overexpress BCRP.16 The relative intracellular imatinib accumulation, measured after a 2-hour exposure to [14C]STI571 in both MCF7/MR (59.5% ± 9.5% [mean ± SD]) and MCF7/AdVp (3.6% ± 1.1%) was significantly lower as compared with MCF7 (Figure 1A). This decreased accumulation is indicative of BCRP-mediated efflux; however, in these drug-selected MCF7 sublines other drug transporters may have been activated. Quantitative real-time RT-PCR (data not shown) and protein data confirmed that only BCRP (Figure 1A, insert), and not MDR1, MRPI, or MRP2 (data not shown), was overexpressed in these MCF7 sublines. Although the difference in imatinib accumulation between MCF7/MR and MCF/AdVp may be explained by the expression data, it should be noted that MCF7/MR overexpresses wild-type (wt)–BCRP whereas MCF/AdVp overexpresses the 482Thr BCRP variant. Notably, mutations at 482Arg of wt-BCRP have been implicated in substrate specificity.15 Therefore, BCRP-mediated transport of imatinib was studied in HEK293 cells transfected with BCRP variants, that is, 482Arg (HEK293/R), 482Gly (HEK293/G), 482Thr (HEK293/T), or empty vector (HEK293/Neo). Similar to MCF7, overexpression of BCRP in HEK293 cell lines resulted in a markedly decreased imatinib uptake (Figure 1B), confirming that imatinib is a substrate for wt-BCRP and both mutant (mt)–BCRP variants. However, the existence of small differences in imatinib accumulation between wt- and mt-BCRP cannot be excluded. Such subtle differences were also observed for mitoxantrone.24 Regarding BCRP-mediated imatinib efflux, our data suggest that codon 482 of BCRP does not play such a pivotal role in substrate specificity as has been reported for methotrexate (MTX) and doxorubicin.17,22-24 Accordingly, we found that doxorubicin is not transported by wt-BCRP, whereas a significant decrease in doxorubicin accumulation (∼40%) was seen in both mt-BCRP variants (Figure 1C). Furthermore, we showed that mitoxantrone is a substrate for both wt- and mt-BCRP (Figure 1D). With respect to this mutational hot-spot at codon 482 of BCRP, imatinib parallels the substrate specificity pattern of mitoxantrone and not that of doxorubicin or MTX.17,23,24

Next, we determined whether the decreased imatinib accumulation could be reversed by Ko-143, a specific inhibitor of BCRP-mediated transport.17 Evidently, Ko-143 could almost completely restore the decreased imatinib accumulation in cells overexpressing wt-BCRP (HEK293/R: 1.7-fold increase in the presence of Ko-143, P < .01 Student t test) as well as in the 2 mt-BCRP variants (HEK293/G: 4.1-fold increase, P < .001; HEK293/T: 2.1-fold increase, P < .01), whereas this specific inhibitor had no effect on the control HEK293/Neo cells (Figure 1E).

Since mitoxantrone is one of the key substrates for BCRP, we determined whether imatinib is a competitive substrate for the same binding site. Mitoxantrone accumulation in the BCRP-positive MCF sublines was clearly increased by addition of 15 μM imatinib as a competitor, whereas accumulation in the BCRP-negative MCF7 parental line was hardly affected (Figure 2A). Similar results were found in BCRP-overexpressing HEK293 cells, that is, mitoxantrone steady-state levels were markedly increased by the addition of imatinib (Figure 2B). Moreover, mitoxantrone efflux was almost completely inhibited by 15 μM imatinib. These competition studies suggest that cotreatment of imatinib with other BCRP substrate drugs may have a significant impact on the oral bioavailability of these drugs.

It was recently shown that imatinib can potently reverse BCRP-mediated resistance to topotecan and SN-38 in vitro; however, in contrast to our results presented here, it was concluded that imatinib directly inhibits BCRP-mediated transport without being a competitive substrate.25 The investigators reported that the...
Figure 2. Effect of imatinib as a competitor for BCRP-mediated efflux of mitoxantrone. (A) Effect of imatinib (15 μM) on mitoxantrone steady-state accumulation (3 μM) in MCF7 parental (left panel), MCF7/MR (middle panel), and MCF7/AdVp (right panel). MCF7 sublines were simultaneously incubated for 2 hours with mitoxantrone (3 μM), with or without imatinib as a competitor (1 μM-15 μM). The fluorescence data of at least 20 000 events per time point are shown and fluorescence intensity (peak signal) of mitoxantrone is expressed in arbitrary units (au). (B) Mitoxantrone steady-state accumulation in the presence of 15 μM imatinib in HEK293 cells. Representative results from 3 independent experiments are shown. Relative accumulation data (mean ± SD) are given as a fraction (%) relative to the accumulation found in HEK293 control cells without imatinib.

accumulation and subsequent efflux of 14C-labeled imatinib was similar in cells expressing functional or nonfunctional BCRP. The apparent discrepancy with our findings may be ascribed to methodologic differences, among which are substantial dissimilarities in incubation time and temperature. Evidently, our accumulation data (Figure 1A-B) and in particular the results of the BCRP-specific inhibitor Ko-143 (Figure 1E) are consistent with the fact that imatinib is a substrate of BCRP. Together, our results indicate that BCRP can function as an active outward transport mechanism for imatinib and this drug efflux pump may therefore play an important role in drug-drug interaction and cellular resistance to imatinib.

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