Nilotinib and imatinib inhibit cytarabine cellular uptake: Implications for combination therapy

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The tyrosine kinase inhibitor (TKI) imatinib has been used for a decade to treat chronic myeloid leukemia (CML). A very efficient response is obtained with patients in chronic phase, but its efficacy in late phase patients is often transient. The combination of imatinib or of the new TKI nilotinib with cytarabine is a new treatment approach proposed for CML. We have investigated the effect of imatinib and nilotinib on cytarabine uptake, and have found that both molecules inhibit cytarabine transport. These results should impact on the design of clinical trials that investigate the combination of TKIs and nucleoside analogs.

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1. Introduction

Chronic myeloid leukemia (CML) is characterized by a reciprocal translocation between chromosomes 9 and 22. This genetic modification leads to the generation of the bcr-abl fusion gene that encodes a chimeric protein with constitutive tyrosine kinase activity [1]. Imatinib (Gleevec) is a very efficient inhibitor of the Bcr-Abl fusion protein that has been used for a decade to treat CML patients and acute lymphoblastic leukemia (ALL) patients that harbor a very similar chromosome translocation. An efficient and sustained response to imatinib is obtained in patients with CML in chronic phase but its efficacy is generally transient in late stages CML (accelerated and blast crisis phases) [2–4]. Moreover, imatinib is not an optimal treatment for 30–35% of the patients in chronic phase; some patients have to discontinue treatment due to side effects, and others lose or do not achieve a complete cytogenetic response, which significantly increases the risk of disease progression [2]. Thus, new therapeutic approaches that increase the cytogenetic response would be beneficial to CML patients.

Nilotinib (Tasigna) is a rationally designed Bcr-Abl inhibitor with greater potency and specificity than imatinib [1]. Furthermore, it is active against several Bcr-Abl mutants that are resistant to imatinib [1]. Nilotinib efficacy was first demonstrated in patients who failed imatinib therapy but its superiority over imatinib has been also confirmed in newly diagnosed CML patients [1,5–7].

Treatments that combine imatinib with other chemotherapeutic agents are also actively investigated in CML and ALL patients. The combination of imatinib with the nucleoside analog cytarabine was an obvious regimen to test in the clinic since cytarabine combined to interferon alpha was the previous standard of care for CML before the imatinib era [8], and that several in vitro studies reported synergistic activities with imatinib and cytarabine [9–11]. A similar combination treatment could be envisaged for nilotinib that is also able to synergize with cytarabine [12].

Nucleosides use specific transporters to enter into cells, with the equilibrative nucleoside transporter 1 (ENT1) being the main cytarabine transporter; its low abundance in acute myeloid leukemia blasts correlates with a lack of response to cytarabine treatment [13]. Few years ago it was reported that some p38 MAPK inhibitors could block nucleoside transport, and that this inhibition occurs in a p38 MAPK-independent manner [14]. This finding was later extended to other types of kinase inhibitors that have different protein targets. Imatinib was tested and it was among the most efficient to block nucleoside transport; it inhibited uridine and thymidine transport by almost 70% at a 10 μM concentration [15]. In another study, it was also reported that imatinib would inhibit the uptake of the nucleoside analog fludarabine with a similar order of magnitude [16].

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We then hypothesized that a combination treatment with cytarabine and imatinib or nilotinib would not be optimal for the treatment of CML if both drugs were used simultaneously. Thus, we investigated to which extent imatinib and nilotinib would inhibit the cellular uptake of the natural nucleoside thymidine, and the nucleoside analog cytarabine in CML cells.

2. Materials and methods

2.1. Cell lines and cell culture

K562 (CCL-243) and MEG-01 (CRL-2021) cell lines were obtained from ATCC. Both cell lines were cultured in RPMI-1640 (Wisent, St-Bruno, Canada) supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin at 37 °C in a humidified atmosphere with 5% CO₂.

2.2. Uptake assays of [³H]-thymidine and [³H]-cytarabine

K562 or MEG-01 cells (4 × 10⁶ cells per sample) were incubated at 37 °C for 15 min with 5 µM imatinib or nilotinib at different concentrations, in 0.5 ml of transport buffer (20 mM Tris/HCl, 3 mM K₂HPO₄, 1 mM MgCl₂, 6H₂O, 2 mM CaCl₂, 5 mM glucose, and 130 mM NaCl, pH 7.4). An additional 0.5 ml of transport buffer containing 1 µCi of radiolabeled thymidine ([²⁷]Cl/mmol; Perkin Elmer, Waltham, MA) or cytarabine ([³¹]Cl/mmol; Moravek Biochemicals, Brea, CA) was then added for an additional 5 min in presence of imatinib or nilotinib (Selleckchem, Houston, TX). Cells were then washed 3 times with cold transport buffer containing 1 mM cold thymidine or cytarabine. The cell pellets were lysed with 10% SDS and radioactivity was quantified with a beta counter. Controls in these experiments were treated with the DMSO alone, that is the solvent used to dissolve the drugs, and dipyridamole, a well-known inhibitor of ENT1 and ENT2.

3. Results

3.1. Imatinib inhibits thymidine and cytarabine uptake in CML cells

The effect of imatinib on nucleoside transport was first examined with thymidine. At a 10-µM concentration, imatinib was able to inhibit the entry of radiolabeled thymidine in K562 cells by 76%. Imatinib was still efficient and blocked by 43% the entry of thymidine at 1 µM, but its effect on thymidine transport at 0.1 µM was not significant (Fig. 1a). With the MEG-01 CML cell line, thymidine entry was inhibited by 72 and 52% with 10 and 1 µM imatinib, respectively. As with K562 cells, the effect at 0.1 µM was not significant with the MEG-01 cell line (Fig. 1b). A very similar trend toward cytarabine transport was also found for imatinib. In K562 cells, cytarabine uptake was inhibited by 50% at a 10-µM concentration of imatinib. The inhibition of cytarabine entry was less pronounced with 1 µM imatinib (23%) but it was still significant. As for thymidine, the effect on cytarabine transport was not significant with 0.1 µM imatinib (Fig. 1c). With MEG-01 cells, cytarabine entry was inhibited by 78% at a 10-µM concentration of imatinib. The inhibition of cytarabine uptake observed at 1 and 0.1 µM imatinib was not significant (Fig. 1d).

3.2. Nilotinib is a more potent inhibitor than imatinib for nucleoside cellular entry

Nilotinib was much more potent than imatinib to inhibit nucleoside transport. It prevented the uptake of thymidine in K562 cells by 97% at 10 µM, a level that was similar to the one obtained with the vasodilator molecule dipyridamole. Nilotinib was also very efficient at 1 and 0.1 µM; it blocked the entry of thymidine by 90 and 74%, respectively (Fig. 2a). With the MEG-01 cell line, nilotinib was also extremely potent and blocked the entry of thymidine by 96, 92 and 60% with 10, 1 and 0.1 µM nilotinib, respectively (Fig. 2b). A similar effect of nilotinib was also found on cytarabine transport; at a 10-µM concentration, nilotinib inhibited the entry of cytarabine in K562 cells by 94%. Nilotinib was still very efficient at lower concentrations; it blocked cytarabine transport by 85 and 33% at 1 and 0.1 µM concentrations, respectively (Fig. 2c). A similar trend was also obtained with the MEG-01 cell line; cytarabine

Fig. 1. Inhibition of thymidine (a and b) and cytarabine (c and d) uptake with imatinib. K562 cells (a and c) and MEG-01 cells (b and d) were incubated at 37 °C for 15 min with imatinib transport buffer, and then incubated with 0.5 µCi of [³H] thymidine or [³H] cytarabine for an additional 5 min in presence of imatinib. Cells were then washed 3 times, lysed and radioactivity associated to cell pellets was quantified. DMSO, dimethylsulfoxide; DPD, dipyridamole. Data are expressed as a percentage relative to the samples incubated with DMSO, and are the average of three experiments ± SEM. Statistical significance was assessed by one-way ANOVA followed by Dunnett’s multiple comparison posttest. The values of each column in the histogram are compared to those obtained with the DMSO treatment ( ns: p > 0.05, *p < 0.05, **p < 0.01).
uptake was inhibited by 96, 89 and 54% at 10, 1 and 0.1 μM nilotinib concentration, respectively (Fig. 2d).

4. Discussion

At a common regimen of 400 mg daily, imatinib reaches a mean peak concentration of 4.6 μM and a mean serum trough concentration of 1.5 μM [1]. We report that 1 μM imatinib inhibits cytarabine by 23% in K562 cells, and by 25% in MEG-01 cells (Fig. 1c and d). These results are in line with those reported previously with uridine in K562 cells and fludarabine with human T-lymphocytes [15,16]. Results from a French clinical trial and from the HOVON-51 study that investigate the upfront combination therapy of imatinib and cytarabine showed a high rate of complete hematologic and molecular responses [9,17]. We propose that a different regimen using both drugs (sequentially instead of simultaneously) could even lead to a better outcome. The mean peak concentration of nilotinib is 3.6 μM and the mean serum trough level at the steady state is 1.7 μM at the common regimen of 400 mg twice daily [5]. Based on our results, cytarabine uptake would be inhibited at these values by more than 85% (Fig. 2c and d), and therefore a combination treatment would not be optimal if both drugs are given simultaneously. A sequential regimen would be difficult to implement with nilotinib due to its potent inhibitory effect on nucleoside entry at low concentrations. With a 15-h half-life [5], several days off therapy would be required to decrease the nilotinib concentration below 0.1 μM before cytarabine administration. Such discontinuation of nilotinib treatment would most likely impact on the outcome of a sequential treatment regimen and would have to be carefully evaluated. In any case, only a clinical trial could ascertain the validity of these assumptions.

Several in vitro studies described a synergistic or an additive effect between imatinib and cytarabine or nilotinib and cytarabine that seem to contradict our results and those from others who reported an inhibition of nucleoside uptake with imatinib or nilotinib [10,11,15,16,18]. In the former studies, the highest doses of imatinib used were 0.8 or 0.25 μM [10,11,18]. These doses are below the mean serum concentration of imatinib and are not very efficient for inhibiting cytarabine cellular uptake (Fig. 1c and d). The additive/synergistic effect that was reported with nilotinib was only observed with LAMA84 and not with K562 cells [12]. Furthermore, the highest dose of nilotinib used in the latter study was 0.1 μM, which corresponds to the lowest dose used in our experiments leading to less than 50% inhibition of cytarabine uptake (Fig. 2c and d).

The exact mechanism of nucleoside transport inhibition by imatinib and nilotinib has not been investigated in this work. In a previous study, the down-regulation of ENT1 mRNA expression by imatinib led to the inhibition of uridine uptake in a mouse cell line [19]. A similar mechanism cannot account for our results because of the different timeframes. The inhibitory effect was only observed after a 12-hour incubation period with imatinib in the mouse cells. However, the inhibition occurs in less than 5 min in our experiments, suggesting that imatinib and nilotinib act by direct binding to ENT1, as it has been suggested for other tyrosine kinase inhibitors [14].

Overall, we have demonstrated that nilotinib and imatinib can inhibit cytarabine transport in two CML cell lines at concentrations achievable in the sera of patients. This study has important practical implications that can impact on the design and outcome of future clinical trials that investigate the therapeutic value of combination of kinase inhibitors and nucleoside analogs. The underlying message is that kinase inhibitors should be tested for their capacity to inhibit nucleoside transport beforehand.

Conflict of interest statement

The authors declare no conflict of interest in connection with this work.
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Contributions. JBS, KG and MC performed experiments, and PoDel and MC designed the study and wrote the manuscript.

References