Imatinib Affects the Expression of SLC22A1 in a Non-Linear Concentration-Dependent Manner Within 24 Hours

Background: Imatinib is actively transported into cells by the organic cation transporter (OCT1), encoded by SLC22A1. As a result, the expression of SLC22A1 is considered a prognostic marker for treatment with imatinib in patients with chronic myeloid leukemia (CML). Although limited, there is conflicting evidence indicating that imatinib may affect the expression of SLC22A1. However, thus far, no studies have investigated the effect of imatinib on SLC22A1 expression in an imatinib-sensitive cell line, which would mimic a typical clinical setting. Changes in the expression of SLC22A1 as a result of imatinib could potentially negate its usefulness as a prognostic marker.

Material/Methods: The K562 CML cell line was exposed to varying concentrations of imatinib for 24, 48, and 72 h and SLC22A1 expression was determined by quantitative real-time PCR.

Results: Our findings suggest that imatinib affects the expression of SLC22A1 within 24 h in a non-linear concentration-dependent manner.

Conclusions: This is the first study to report on the short-term effect of imatinib on the expression of SLC22A1 in an imatinib-sensitive CML cell line. Our results suggest that imatinib affects SLC22A1 mRNA expression in a non-linear dose-dependent manner and that the changes in the expression of SLC22A1 as a result of the concentration of imatinib occur within 24 h of exposure to imatinib and remain stable thereafter for up to 72 h.

MeSH Keywords: Gene Expression • Leukemia, Myelogenous, Chronic, BCR-ABL Positive • Organic Cation Transporter 1

Full-text PDF: https://www.basic.medscimonit.com/abstract/index/idArt/909124
**Background**

Despite the clinical success of imatinib in treating patients with chronic myeloid leukemia (CML), approximately 25% of patients fail to achieve an optimal response due to inadequate BCR-ABL inhibition as a result of decreased intracellular accumulation of imatinib [1]. Imatinib is actively transported across the cell membrane by the organic cation transporter (OCT1), encoded by SLC22A1 [2]. Although there is consensus that OCT1 is a key determinant of the intracellular levels of imatinib achieved in cells, there is conflicting data regarding its usefulness as a prognostic marker.

A limited number of studies have reported contradictory results on the possible effect of imatinib on the expression of SLC22A1 [3–8], thus negating its usefulness as a prognostic marker for CML patients on imatinib therapy. Some studies suggest that SLC22A1 expression is significantly higher in CML patients responding favorably to imatinib compared to patients at diagnosis, thus suggesting that imatinib may have a possible induction effect on the expression of SLC22A1 [3,4]. However, other studies have either reported no correlation in the expression of SLC22A1 in CML patients treated with imatinib for responders compared to non-responders [5], or that there is considerable variation in the expression of SLC22A1 among responders and non-responders alike [6]. One study has even reported a decrease in SLC22A1 expression in some imatinib-resistant CML patients after therapy was commenced [7]. In an in vitro study, the K562 CML cell line was exposed to a low dose of imatinib which was serially increased as the cell line became more resistant [8]. The authors observed an increase in SLC22A1 expression when the cell line became sequentially resistant to 0.25 μM, 0.5 μM, 1.0 μM, 2.0 μM, and 5.0 μM at 105 days up to 393 days of exposure [8]. The latter study concluded that imatinib affected the expression of SLC22A1 in a linear, concentration-dependent manner over time for between 105 and 393 days [8]. However, it is uncertain whether an imatinib-resistant CML cell line would mimic a typical clinical setting in which most patients would be imatinib-sensitive. Furthermore, the short-term effect of imatinib on SLC22A1 expression is unknown. The aim of this study was to investigate the effect of varying concentrations of imatinib (0.1 μM, 0.2 μM, 0.5 μM, 1.0 μM, 2.0 μM, 5.0 μM, and 10.0 μM) on the expression of SLC22A1 from 24 h up to 72 h in an imatinib-sensitive CML cell line.

**Material and Methods**

**Cell culture**

The human CML cell line, K562, was cultured in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO) with 10% fetal bovine serum (Sigma-Aldrich), 1% penicillin-streptomycin (Sigma-Aldrich), and 5 μg/ml Plasmocin (InvivoGen) at 37°C under a 5% CO₂ atmosphere. A stock solution of 10 mM imatinib (kindly provided by Novartis) was prepared in nuclelease-free water and stored at −70°C. Cells were incubated with 0 μM, 0.1 μM, 0.2 μM, 0.5 μM, 1 μM, 2 μM, 5 μM, and 10 μM of imatinib for 24, 48, and 72 h. The experimental period was limited to 72 h in order to study the short-term effect of imatinib on the expression of SLC22A1. At the end of each time interval, control and experimental cells were counted using the TC10 Automated Cell Counter (BIO-RAD), diluted to a concentration of 2×10⁵ cells/mL.

**Determination of SLC22A1 expression**

Total RNA was extracted from K562 cells using TRI reagent (Sigma-Aldrich) according to the manufacturer’s instructions. The concentration of extracted RNA was determined using the Quant-iT RNA Assay Kit according to manufacturer’s instructions (Invitrogen). A standard amount of 2 μg of total RNA was reverse-transcribed using the High Capacity RNA-to-cDNA Kit (Applied Biosystems) in a final volume of 20 μL according to the manufacturer’s instructions. The expression level of SLC22A1 was evaluated by quantitative real-time polymerase chain reaction (qPCR) on a 7500 FAST qRT-PCR system (Applied Biosystems). The TaqMan Gene Expression Assay Kit was used to perform gene expression of SLC22A1 (Hs 00427555_m1) (Applied Biosystems). GUS was used as the reference gene for quantification. Commercial copy number standards (10⁴, 10⁵, and 10⁶ copies) were used to quantify copies of GUS mRNA (Ipsogen), and serial dilutions (10², 10³, 10⁴, 10⁵, 10⁶ copies) of an Ultramer oligonucleotide synthesized by Integrated DNA Technologies were used to quantify copies of SLC22A1 mRNA as previously described [9]. All assays were performed in triplicate and the entire experiment was repeated independently 3 times. The statistical evaluation of the data was performed using ANOVA and t test with a 95% confidence interval.

**Results**

Interestingly, in this study, we found that imatinib induced SLC22A1 expression in a non-linear dose-dependent manner within 24 h (Figure 1). Although there was an increase in SLC22A1 expression compared to the untreated control at all concentrations of imatinib, the increase was not always statistically significant.

Additionally, in order to determine the effect of short-term exposure to imatinib on the expression of SLC22A1, cells were exposed to varying concentrations of imatinib for 24, 48, and 72 h. We found that the expression of SLC22A1 did not change significantly following treatment with imatinib after 24 h (p=0.33), 48 h (p=0.27), or 72 h (p=0.29), but instead was dependent on the concentration of imatinib.

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)
Discussion

The findings in our study indicate that imatinib affects the expression of SLC22A1 in a non-linear dose-dependent manner which is time-independent for up to 72 h. These results contradict previous findings that reported a linear correlation between SLC22A1 expression and imatinib concentration in a study on the development of resistance to imatinib [8]. A previous study [8] determined SLC22A1 expression at the time of the K562 cell line becoming sequentially resistant to increasing concentrations of imatinib (0.25 μM at 105 days up to 5.0 μM at 253 days). We suggest that the latter does not determine the effect of imatinib concentration on the expression of SLC22A1 in imatinib-sensitive cells.

The non-linear increase in SLC22A1 expression to increasing concentrations of imatinib may explain the heterogeneous expression of SLC22A1 observed in CML patients [5–7]. It has been reported that there is substantial inter-patient variability in plasma levels of imatinib achieved between patients on the same dose of the drug [10,11]. A more recent study [11] did not find a significant linear correlation between OCT1 protein expression and plasma levels of imatinib in patients. The lack of linear correlation can be explained from the results of the present study, which demonstrate that imatinib has a non-linear concentration-dependent effect on the expression of SLC22A1.

Furthermore, we found that SLC22A1 expression was unchanged after 24 h of exposure to imatinib for up to 72 h. These findings are similar to those of Engler [12] who reported that the expression of SLC22A1 became stable within 24 h in a promyelocytic HL-60 cell line treated with 2.0 μM imatinib for 24 h and 7 days. As we previously commented, the change in SLC22A1 expression reported in imatinib-resistant K562 cells for up to 393 days [8] does not appear to be characteristic of an imatinib-sensitive cell line. Thus, we conclude that in an imatinib-sensitive cell line, imatinib affects the expression of SLC22A1 within 24 h of exposure and remains stable thereafter.

Studies have reported a lack of correlation between SLC22A1 expression (at the mRNA and protein level) and SLC22A1 functional activity as measured by the cellular uptake of imatinib. Various theories have been proposed to explain this phenomenon, including: (1) The potential need for transporters to up regulate when challenged with various drugs [13]; (2) The expression of SLC22A1 may be a composite surrogate for the expression of other transporters that may affect the intracellular uptake and retention of imatinib [14]; and (3) Imatinib may exhibit potent inhibitory effects on SLC22A1 function [15]. The findings from the present study support the suggestion [14] that the expression of SLC22A1 does not represent SLC22A1 transport function, and we hypothesize that other rate-limiting physiological factor(s) may influence the cellular uptake of imatinib.

Conclusions

In conclusion, to the best of our knowledge, this is the first study to demonstrate that there is a non-linear concentration-dependent effect of imatinib on SLC22A1 expression in an imatinib-sensitive cell line. Furthermore, imatinib affects the expression of SLC22A1 within 24 h and remains stable for up to 72 h. Finally, these data support the suggestion that the transport activity of SLC22A1 is not dependent on just SLC22A1 expression levels, but also depends on other currently unknown rate-limiting physiological factor(s). The findings from this study complete the knowledge gap on the short-term effect of imatinib on the expression of SLC22A1 in an imatinib-sensitive CML cell line.

Acknowledgements

The authors would like to thank Novartis for providing us with the imatinib used in this study.
References:

1. Engler JR, Hughes TP, White DL: OCT-1 as a determinant of response to antileukemic treatment. Clin Pharmacol Ther, 2011; 89: 608–11

2. Thomas J, Wang L, Clark RE, Pirmohamed M: Active transport of imatinib into and out of cells: Implications for drug resistance. Blood, 2004; 104: 3739–45

3. Bazeos A, Marin D, Reid AG et al: hOCT1 transcript levels and single nucleotide polymorphisms as predictive factors for response to imatinib in chronic myeloid leukemia. Leukemia, 2010; 24: 1243–45

4. Engler JR, Zannettino ACW, Bailey CG et al: Oct-1 function varies with cell lineage but is not influenced by BCR-ABL. Haematologica, 2011; 96: 213–20

5. Malhotra H, Sharma P, Malhotra B et al: Molecular response to imatinib & its correlation with mRNA expression levels of imatinib influx & efflux transporters in patients with chronic myeloid leukemia in chronic phase. Indian J Med Res, 2015; 142: 175–82

6. Gromicho M, Magalhães M, Torres F et al: Instability of mRNA expression signatures of drug transporters in chronic myeloid leukemia patients resistant to imatinib. Oncol Rep, 2013; 29: 741–50

7. Kim Y-K, Lee S-S, Jeong S-H et al: OCT-1, ABCB1 and ABCG2 expression in imatinib-resistant chronic myeloid leukemia treated with dasatinib or nilotinib. Chonnam Med J, 2014; 50: 102–11

8. Gromicho M, Dinis J, Magalhães M et al: Development of imatinib and dasatinib resistance: Dynamics of expression of drug transporters ABCB1, ABCG2, MVP, and SLC22A1. Leuk Lymphoma, 2011; 52: 1980–90

9. Vlijoen CD, Thompson GG, Sreenivasan S: Stability of Ultramer as copy number standards in real-time PCR. Gene, 2013; 516: 143–45

10. Larson RA, Druker BJ, Gulhhot F et al, for the IRIS (International Randomized Interferon vs. STI571) Study Group: Imatinib pharmacokinetics and its correlation with response and safety in chronic-phase chronic myeloid leukemia: A subanalysis of the IRIS study. Blood, 2008; 111: 4022–28

11. Cao C, Li X, Liu T et al: Human organic cation transporter 1 protein levels of granulocytes can optimize imatinib therapy in patients with chronic myeloid leukemia. Acta Haematol, 2015; 133: 199–204

12. Engler J: Cell lineage, cell maturity and BCR-ABL factors which influence imatinib uptake in chronic myeloid leukaemia. http://hdl.handle.net/2440/70451. Accessed 06 November 2012

13. White DL, Dang P, Engler J et al: Functional activity of the OCT-1 protein is predictive of long-term outcome in patients with chronic-phase chronic myeloid leukemia treated with imatinib. J Clin Oncol, 2010; 28: 2761–67

14. Hu S, Franke RM, Filipski KK et al: Interaction of imatinib with human organic ion carriers. Clin Cancer Res, 2008; 14: 3141–48

15. Minematsu T, Giacomini KM: Interactions of tyrosine kinase inhibitors with organic cation transporters and multidrug and toxic compound extrusion proteins. Mol Cancer Ther, 2011; 10: 531–39