

MEASUREMENT AND MODULATION OF TYROSINE KINASE INHIBITORS IN CHRONIC MYELOID LEUKAEMIA (CML)

Researchers

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Aim

Tyrosine kinase inhibitors (TKI) such as imatinib and nilotinib have revolutionised CML patient treatment. However, whilst very successful in controlling the disease, TKI fail to cure CML because there is a small percentage of the leukaemic cells that is resistant or insensitive to these drugs and remains dormant in the patient; so if drug treatment was stopped, leukaemic cells may be reactivated and the disease would return. We propose that such cells are not eliminated due to insufficient levels of the drug inside the cells. One way in which drug levels can be mediated is via pumps on the surface of the cells known as Multidrug Resistance (MDR) proteins, that can import or export drugs including TKI. We have recently found that a number of these pumps are increased in CML cells. As a result, we aimed:

1. To determine the intracellular drug concentrations in stem cells treated *in vitro* with TKI.
2. To identify whether the expression of MDR proteins is likely to affect the intracellular levels of these drugs in CML stem cells and whether modulation of MDR proteins may further increase the efficacy of TKI.

Project Outline/Methodology

Blood samples were obtained from patients in the early stage (chronic phase, CP) of CML and non-CML patients as controls. Stem cells were selected based on their cell surface expression of CD34 and CD38 proteins and separated into groups of CD34⁺38⁻, CD34⁺38⁺, CD34⁻38⁺ and total CD34⁺ cells. CML patient cells were treated *in vitro* with increasing concentrations of TKI approximating the concentrations measured in blood samples of patients treated with the different TKI. The intracellular concentrations of TKI were measured by high performance liquid chromatography (HPLC). The role of MDR in CML was investigated by direct measurement of gene expression by RT-PCR, as well as substrate displacement assays to verify the interaction of TKI with MDR as substrates or inhibitors of the pumps.

Key Results

A significant difference in the intracellular concentration of all TKI was observed, with the primitive CML CD34⁺38⁻ cells accumulating less drug than the total CD34⁺ population. Imatinib and nilotinib were found to differentially interact with MDR proteins; imatinib is an inhibitor of ABCG2

and a weak inhibitor of MDR1 but has no interaction with MRP1 while nilotinib is an inhibitor of ABCG2 and MDR1, and exerts a weak inhibitory effect on MRP1 function. The effect of transporter inhibitors combined with TKI on undivided CD34⁺ and more mature CD34⁺ cells from CML patients in CP were assessed with regard to cell division, apoptosis and BCR-ABL kinase activity. In keeping with their inhibitory activity, neither imatinib nor nilotinib demonstrated significantly increased efficacy when combined with specific transporter inhibitors (FTC for ABCG2 or PSC 833 for MDR1). Therefore concentration and activity of imatinib or nilotinib cannot be altered by the activity of these common MDR proteins.

Conclusions

The low intracellular levels of TKI in the dormant leukaemic stem cells can partly explain why treatment is failing to eradicate this population. However, this is unlikely to be due to the activity of MDR efflux transporters.

What does this study add to the field?

This study provides evidence that MDR efflux transporters are not contributing to TKI-insensitivity of primitive CML stem cells.

Implications for Practice or Policy

Whilst this laboratory based study will not directly affect NHS practice or policy, it is relevant to understand why drug therapies fail to be curative in major diseases such as cancer, and how drug efficacy may be augmented ultimately to improve the patients' outcome.

Where to next?

We are interested in exploiting the use of a potential novel drug delivery system to increase intracellular drug levels in target cells which may sensitize these primitive cells to TKI treatment.

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