Abstract

Chronic Myeloid Leukemia (CML) is associated with the BCR/ABL fusion gene which encodes a constitutively-active protein tyrosine kinase, leading to a deregulation of tyrosine kinase activity. The BCR/ABL tyrosine kinase inhibitor, imatinib, is the frontline therapy for CML. However, imatinib-acquired resistances still occurs from the mutations. Therefore, a need to find alternative ways to kill imatinib resistant cells is in order. This study shows that Purvalanol A, a CDK 2 inhibitor induced apoptosis in imatinib-resistant cells by inducing turnover of the anti-apoptotic protein, Mcl-1. This study suggests that Purvalanol A may provide an alternative method to overcome the imatinib resistance in CML and provide a new strategy for further study in the resistance mechanisms.

Background

About Chronic Myeloid Leukemia (CML)

Increased and unregulated growth of myeloid cells in bone marrow and in blood.

Results from single genetic abnormality (Philadelphia (Ph) chromosome), a t(9;22) reciprocal translocation generating a BCR/ABL fusion gene. This encodes a constitutively-active protein tyrosine kinase, involved in dysregulated cell proliferation, apoptosis, differentiation and adhesion (A) 1.

CML treatment and drug resistance

The tyrosine kinase inhibitor (TKI), imatinib, binds to ATP binding site of BCR- ABL, and is frontline therapy. However, imatinib acquired resistance occurs.

A need to find alternative ways to kill CML cells

Other kinase inhibitors may induce apoptosis in TKI-resistant CML cells Purvalanol A, a human cyclin dependent kinase 2 (CDK 2) inhibitor, induces reversible arrest in G1 phase of the cell.2

Aims

Aims of the project

To determine the effects of purvalanol A on apoptosis of TKI-sensitive and -resistant CML cells

Hypothesis

Purvalanol A may provide an adjunct to TKIs in the treatment of CML.

Materials and Methods

Cell Culture

Two CML cell lines were used: LAMA84 (imatinib-sensitive)3 and KCL-22 (imatinib- resistant).4 They were incubated with imatinib alone, or in combination with purvalanol A.

Cell Viability Assay

Viability was determined by flow cytometry using the Viacount assay, which measures the permeability of the plasma membrane.

Immunoblotting

The levels of expression anti apoptotic protein, Mcl-1 and a range of pro-and anti-apoptotic proteins were measured by Western blotting after incubation in the presence and absence of imatinib, purvalanol A and cycloheximide.

Immunoblotting

Purvalanol A decreased expression of anti apoptotic Mcl-1 protein in imatinib-resistant cells.

Immunoblotting

The half life of Mcl-1 in KCL-22 cells is decreased by Purvalanol A

Conclusions

- Purvalanol A decreased viability and induced apoptosis in imatinib-resistant CML cells.
- Imatinib treatment of LAMA84 cells resulted in a large decrease in the levels of Mcl-1, but not in KCL-22 cells, in line with the effects of this drug on apoptosis.
- Purvalanol A induced apoptosis and caused a large decrease of Mcl-1 level in imatinib-resistant KCL-22 cells. Both drugs had very little effect on expression of other anti-apoptotic proteins, such as Bcl-2 and Bcl-X, or pro-apoptotic proteins such as Bak in either cell lines (results not shown).
- The half life of Mcl-1 in imatinib-resistant CML cells was decreased by Purvalanol A Purvalanol A may provide an adjunct to TKIs in the therapy of CML by inducing turnover of the anti-apoptotic protein, Mcl-1.


The effects of the CDK 2 inhibitor, Purvalanol A in combination with tyrosine kinase inhibitors, imatinib in imatinib-sensitive and -resistant chronic myeloid leukaemia cell lines.

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