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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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 important. 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.

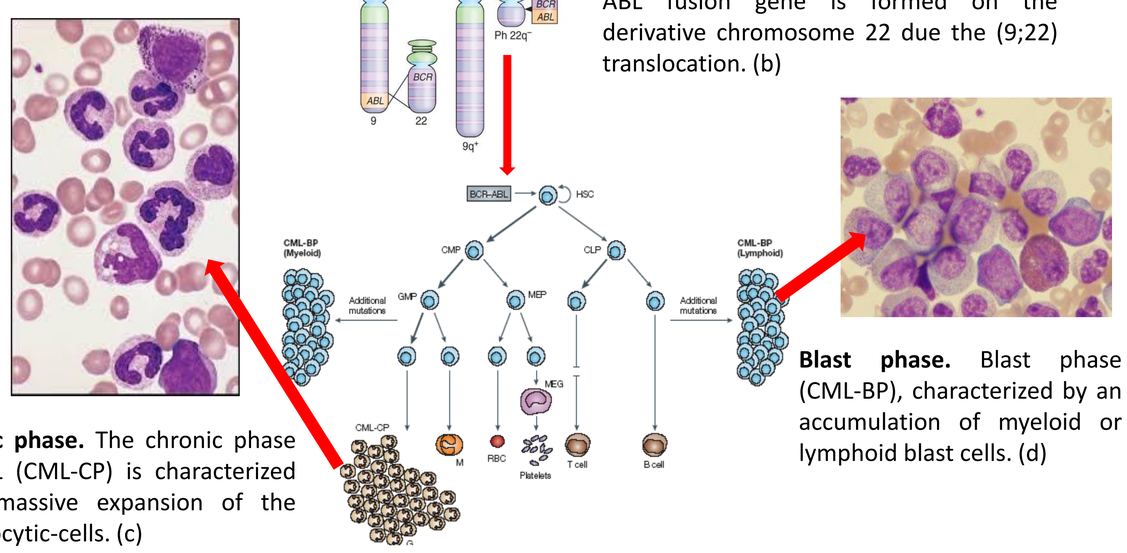
Background

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].

A



Philadelphia Chromosome. A BCR-ABL fusion gene is formed on the derivative chromosome 22 due the (9;22) translocation. (b)

Chronic phase. The chronic phase of CML (CML-CP) is characterized by a massive expansion of the granulocytic-cells. (c)

Blast phase. Blast phase (CML-BP), characterized by an accumulation of myeloid or lymphoid blast cells. (d)

A. The development of chronic myelogenous leukaemia. CML is developed by expression of the *BCR–ABL* fusion gene product in haematopoietic stem cells (HSCs). HSCs can differentiate into common myeloid progenitors (CMPs) and lymphoid progenitors (CLPs). These cells then differentiate into different types of circulating cells. (Granulocyte/macrophage progenitors (GMPs); progenitors of granulocytes (G) and macrophages (M); megakaryocyte/erythrocyte progenitors (MEPs; progenitors of red blood cells (RBCs) and megakaryocytes (MEGs)). (a)

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[2].

A need to find alternative ways to kill resistant 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[3].

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[4,5]) and KCL-22 (imatinib- resistant[6,7]). 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.

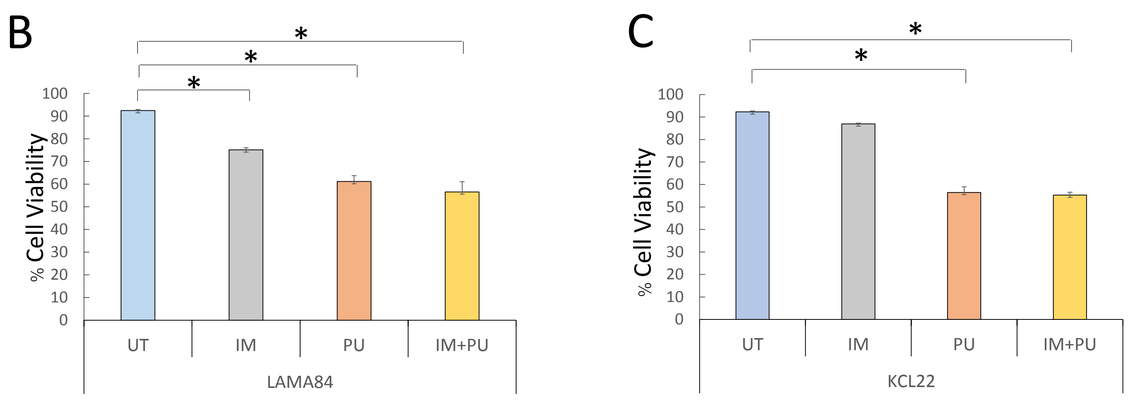
Immunoblotting

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.

Results

Cell Viability: Purvalanol A can decrease cell viability in imatinib resistant CML cells

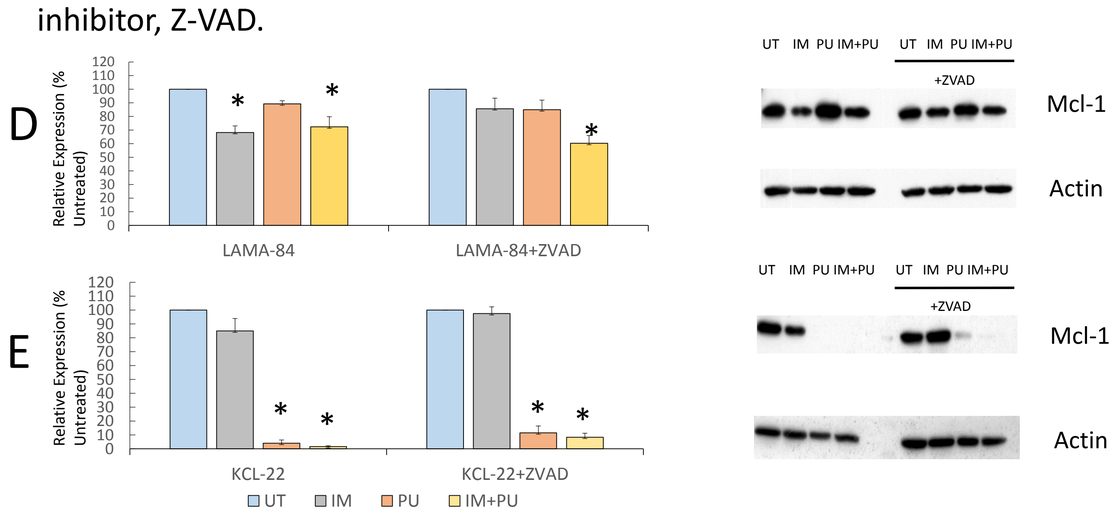
Imatinib (10 µM) induced apoptosis in LAMA84 cells within 24 h of incubation, whereas this drug had little effect on the viability of KCL-22 cells under the same conditions. Purvalanol A (at 30 µM) resulted in a rapid (within 24h) decrease in cell viability of both imatinib-sensitive LAMA84 cells and imatinib-resistant KCL-22 cells



Differential sensitivity of CML cells to imatinib, purvalanol A. Both LAMA84 (B) and KCL-22 (C) were incubated for 24 in the absence (UT) and presence (IM), (PU), and (IM+PU) of imatinib (10 µM), Purvalanol A (30 µM) or both inhibitors. Viability was assessed by flow cytometry. Data are shown as a % of viable cells (±SEM, n=3), * = p≤0.05.

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

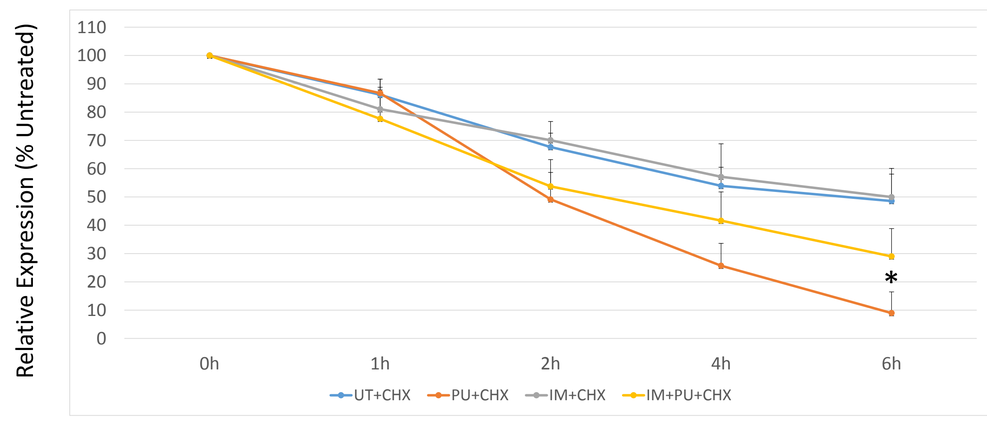
Imatinib treatment of LAMA84 cells resulted in a significant decrease in the levels of the anti-apoptotic protein, Mcl-1, but had very little effect on KCL-22 cells.
Purvalanol A caused a significant decrease in the levels of expression of Mcl-1 in KCL22 but not LAMA-84. This was not affected by treatment of cells with the pan-caspase inhibitor, Z-VAD.



Effect of imatinib and Purvalanol A on expression of Mcl-1. LAMA-84 (D) and KCL-22 (E) cells were incubated for 24 h in the absence (UT) and presence (IM) of imatinib (10 µM) or (PU) purvalanol A (30 µM), ± ZVAD (20 µM), and samples were prepared for Western blotting for Mcl-1, quantified by densitometry. Data expressed as a % of untreated samples (±SEM, n=3), * = p≤0.05.

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

The effects of addition of imatinib, purvalanol A on the half life of Mcl-1 in KCL-22 were measured by western analysis. Purvalanol A caused a significant decrease in the half life of Mcl-1 level ($p>0.05$).



F. Effect of imatinib, purvalanol A alone and in combination on the half life of Mcl-1 in KCL-22 cells. KCL-22 cell line were incubated for 10 mins with cycloheximide (CHX) to inhibit protein biosynthesis, prior to 0, 1, 2, 4, and 6 h incubation in absence of drug (UT), presence 10 µM imatinib (IM), of presence of 30 µM Purvalanol A (PU), or presence of both drugs (IM+PU). Cell samples were prepared for Western blotting for the level of Mcl-1, which was quantified by densitometry. Data are expressed as % of untreated samples (±SEM, n=3), * = p≤0.05.

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_L 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 might provide an adjunct to TKIs in the therapy of CML by inducing turnover of the anti-apoptotic protein, Mcl-1.

References & Figure credits: 1. Kurzrock, R., *et al., The molecular genetics of Philadelphia chromosome-positive leukaemias.* N.Engl.J.Med,1998. **319**: p. 990-998. 2. Soverini, S., *et al., Implications of BCR-ABL1 kinase domain-mediated resistance in chronic myeloid leukaemia*. Leukemia Research, 2014. **38**: p.10-20. 3. Villerbu, N., et al., *Cellular effects of purvalanol A: a specific inhibitor of cyclin-dependent kinase activities.* Int J Cancer, 2002. **97**(6): p. 761-9. 4. Seigneurin, D., et al., *Human chronic myeloid leukemic cell line with positive Philadelphia chromosome exhibits megakaryocytic and erythroid characteristics.* Exp Hematol, 1987. **15**(8): p. 822-32. 5. Coutre, P., et al., *Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells through gene amplification.* Blood, 2000. **95**(5): p. 1758-66. 6. Kubonishi, I. and Miyoshi L., Establishment of a Ph1 chromosome-positive cell line from chronic myeloid leukemia in blast crisis. Int J C Cloning, 1983. **1**(2):105-17. 7. Quentmeier, H., et al., BCR-ABL1-independent PI3Kinase activation causing imatinib-resistance. Journal of hematology & Oncology, 2011. **4**(6): p 981-991. a. Ren, R., *Mechanism of BCR-ABL in the pathogenesis of chronic myeloid leukemia.* Nature, 2005. **5**: p.172-183. b. Lydon, N., *Attacking cancer at its foundation.* Nat Med, 2009. **15**(10): p. 1153-7. c. http://www.pathologystudent.com/?p=2159 d. http://ashimagebank.qc.astutetech.com/AssetDetail.aspx?AssetID=6870&AssetType=Asset.