Abstract

A novel derivative of the Cdk inhibitor roscovitine that induces apoptosis in CLL and overcomes stromal cell-mediated protection

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Abstract Body:

Cmpd 5 is a derivative of R-roscovitine (CYC-202, seliciclib) with increased potency that selectively inhibits Cdk2, Cdk5 and Cdk9. In chronic lymphocytic leukemia (CLL) a disease that is addicted to the over-expression of anti-apoptotic proteins for survival, inhibition of Cdk9 by Cmpd 5 reduced phosphorylation of the C-terminal domain of RNA polymerase II and blocked transcription. These actions depleted the intrinsically short-lived anti-apoptotic protein Mcl-1, and induced apoptosis in CLL cells in vitro. Cmpd 5 was about 30 times more potent than its parent compound R-roscovitine with an IC50 of 0.86 µM after a 24-hr incubation. Although cell death was initiated after a 4-hr incubation in a time course study of Cmpd 5-induced apoptosis, the maximum cell death was not reached until 10-12 hr. In addition, it was known that co-culture of CLL cells with the marrow and lymphoid stromal cells may be responsible for resistance to fludarabine therapy. We evaluated Cmpd 5 in overcoming such stromal cell-mediated protection. Cmpd 5 killed the CLL cells similarly in the presence or absence of the human stromal cell line StromaNKtert without toxicity to the stromal cells, whereas the stromal cells clearly protected the CLL cells from the toxicity of fludarabine. Since Mcl-1 is the major target of Cmpd 5 in CLL, and the action of Cmpd 5 relies on the intrinsically rapid turn-over rate of Mcl-1, we compared the protein levels and the half-life of Mcl-1 in CLL cells in the presence or absence of the stromal cell layer. After an overnight incubation of CLL cells with the StromaNKtert cells, there was a 3-4 fold induction of Mcl-1 transcript and protein. However, there was no difference in the Cmpd 5-induced decay rate of either the Mcl-1 mRNA or protein, indicating that stromal cells did not alter the stability of Mcl-1. Therefore, the elevated Mcl-1 level likely indicated increased biosynthesis, rather than enhanced stability. Thus, the activation of the survival pathways and induction of the biosynthesis of Mcl-1 by the stromal cells may protect CLL cells from fludarabine-induced apoptosis but loss of the short-lived anti-apoptotic proteins was not affected, explaining the lack of stromal cell protection for CLL cells from Cmpd 5. Similar time-dependence of cell death induction as well as stromal cell response was also observed with other inhibitors of transcription including flavopiridol, R-roscovitine and Actinomycin D, implying a class effect. Taken together, our data suggested that Cmpd 5 is a promising candidate for clinical development for CLL.
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Introduction

• Chronic lymphocytic leukemia (CLL) is characterized as being dependent on the over-expression of anti-apoptotic proteins for survival.
• In such a biologic context, agents that aim at antagonizing or diminishing the anti-apoptotic proteins would release the pro-death signals to commit cells to apoptosis. This has been a focus of new therapeutics in CLL.
• We have proposed a strategy that directly target this pathogenesis of CLL by inhibiting the synthesis of anti-apoptotic proteins using cyclin dependent kinases (Cdk) inhibitors that block transcription.

Hypothesis

Inhibition of transcription by Compound 5 would preferentially reduce the short-lived anti-apoptotic proteins, which would lead to induction of apoptosis in CLL.

Mechanism of action of Cdk9 inhibitors in CLL

Inhibit Pol II CTD phosphorylation
Inhibit RNA synthesis
Intrinsically short-lived mRNAs, such as Mcl-1, XIAP
Intrinsically short-lived anti-apoptotic proteins

Conclusions

• Inhibition of Cdk9 by Compound 5 reduced phosphorylation of RNA pol II and blocked transcription.
• These actions depleted the intrinsically short-lived anti-apoptotic protein Mcl-1 and XIAP, and induced apoptosis in CLL cells in vitro.
• Compound 5 followed a similar time course of cell death induction as the other transcription inhibitors: cell death was initiated after a 4-hr incubation and reached the maximum at 10-12 hr.
• Compound 5 and other transcription inhibitors overcame stromal cell-mediated protection.
• Stromal cells did not increase the stability of Mcl-1. Thus, the stromal cells may protect CLL cells from fludarabine-induced apoptosis but loss of the short-lived anti-apoptotic proteins was not affected, explaining the lack of stromal cell protection for CLL cells from transcription inhibitors.
• Taken together, our data suggested that Compound 5 is a promising candidate for clinical development for CLL.