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PYRROLO-1,5-BENZOXAZEPINES INDUCE APOPTOSIS IN CHRONIC MYELOID LEUKEMIA (CML) CELLS BY BYPASSING THE APOPTOTIC SUPPRESSOR BCR-ABL

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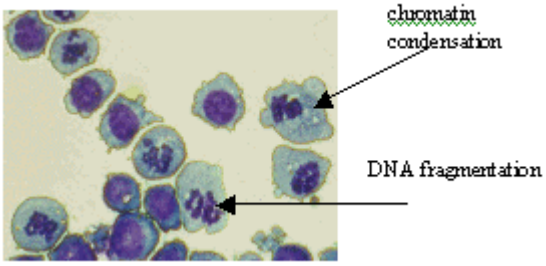
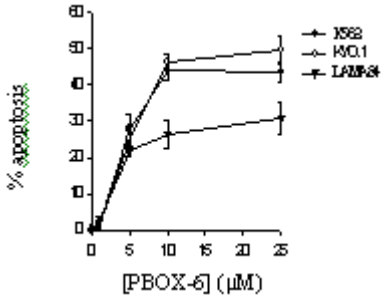
INTRODUCTION. Chronic myeloid leukemia (CML), which accounts for 20% of all leukemias, expresses the transforming oncogene, *bcr-abl*. Expression of *bcr-abl* results in the production of an abnormal tyrosine kinase and is reported to confer resistance against apoptosis induced by many chemotherapeutic agents. Recently a novel series of pyrrolo-1,5-benzoxazepines (PBOXs) were synthesised (Campiani *et al*., 1996) and some of these compounds induce apoptosis in a number of cancerous cells (Zisterer *et al*., 2000). In this study, a number of these novel pyrrolo-1, 5-benzoxazepines were found to induce apoptosis in CML cells. We examined whether Bcr-Abl becomes downregulated and whether its protein tyrosine kinase activity is altered during apoptosis (Mc Gee *et al.*, in press).

METHOD. Cells were cytocentrifuged onto slides and stained with eosin Y and methyl blue. Apoptotic cells were characterised by cell shrinkage, membrane blebbing, nuclear condensation and DNA fragmentation (Fig. 1). Levels of Bcr-Abl expression, protein tyrosine phosphorylation and PARP cleavage were measured by Western blot. Caspase 3-like protease activity was measured using a substrate, Ac-DEVD-AMC, which is cleaved and fluorogenic AMC released.



RESULTS. A representative pyrrolo benxozaxepine, PBOX-6, was found to induce 40-50% apoptosis in CML cells in a time and dose dependent manner (Fig. 2). Downregulation of Bcr-abl was not detected and the tyrosine phosphorylation status of proteins was unchanged up to 24 hours following treatment with PBOX-6. Caspase 3-like proteases were activated in K562

and LAMA 84 cells, but not in KYO.1 cells although apoptosis was induced to the same extent. Pretreatment of cells with a caspase 3-like inhibitor, Z-DEVD-fmk, prior to PBOX-6 inhibited caspase 3-like protease activity, but failed to prevent against apoptosis.



DISCUSSION. We have shown that PBOX-6 is a potent inducer of apoptosis in CML cells and is able to bypass Bcr-Abl mediated resistance. Downregulation of Bcr-Abl did not accompany but rather followed the induction of apoptosis. The tyrosine phosphorylation status of proteins remained unchanged up to 24 hours following treatment with PBOX-6. These results suggest that a reduction in Bcr-Abl expression, or inhibition of tyrosine kinase activity is not the only mechanism by which cells can escape the anti-apoptotic effect of the *bcr-abl* gene. Activation of caspase 3-like proteases is not required for the induction of apoptosis by PBOX-6 in the CML cells examined. Results from this study suggest the potential of this compound as a novel anti-cancer agent for the treatment of CML.

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