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

Margaret M. Mc Gee*, Giuseppe Campiani, Anna Ramunno, Caterina Fattorusso, Vito Nacci, Mark Lawler, D. Clive Williams, Daniela M. Zisterer

Department of Biochemistry, Trinity College, Dublin 2, Ireland
* mmcgee@tcd.ie

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 benxoxazepine, 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
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 \textit{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.

ACKNOWLEDGEMENTS. This study was supported by BioResearch Ireland, National Pharmaceutical Biotechnology Centre.

REFERENCES.