Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol10Issue4abst181P.pdf

Bruton's Tyrosine Kinase (BTK) Inhibition Enhances Chemotherapy in Human Multiple Myeloma

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Bruton tyrosine kinase (Btk) is essential in the development and function of B cells through normal B cell receptor signaling, In B-cell development the receptor for B cell-activating factor (BAFF) of the TNF family (BAFF-R) is coupled to the NF-κB pathway by BTK. Thus, BAFF-induced signaling to NF-κB via BTK serves to promote B-cell survival. As the NF-κB pathway is central to myeloma cell survival, and BTK couples cell surface survival signals to the NF-κB pathway in B-cell development, as well as the BTK inhibitor ibrutinib (previously known as PCI-32765) being highly effective in killing chronic lymphocytic leukaemia cells, we therefore investigated the effects of BTK inhibition in multiple myeloma (MM).

MM cells were obtained from the bone marrow of 8 previously untreated patients under ethical approval (LRECref07/H0310/146). After initial purification, MM samples with <80% cells expressing CD138, were purified using a CD138+ selection kit (Miltenyi Biotec). MM cell lines (RPMI8226, U266, LP1, MM1S, MM1R and H929) were obtained from the European Collection of Cell Cultures where they are authenticated by DNA-fingerprinting. MM cells and monocytes were treated with different doses of ibrutinib, LFM-A13, bortezomib or lenalidomide then viable numbers measured with Cell-Titre GLO (Promega). Flow cytometry was used for measuring apoptosis was performed on the Accuri C6 flow cytometer (Accurri). Samples were collected and stained with Annexin- V and Propidium Iodide (PI), followed by detection. RNA and protein were extracted to analyse mRNA and protein regulated by BTK inhibition using real-time PCR and Western blotting.

Here we show that the BTK inhibitors ibrutinib and LFM-A13, are able to enhance the effects of the multiple myeloma cytotoxic agents bortezomib and lenalidomide. Ibrutinib was shown to be cytotoxic to multiple myeloma cell lines and towards malignant plasma cells from patients with multiple myeloma. The co-treatment of these cell types with ibrutinib significantly augments the cytotoxic activities of bortezomib and lenalidomide chemotherapies. The cytotoxicity of ibrutinib in multiple myeloma is mediated via its inhibition of the nuclear factor-κB (NF-κB) pro-survival signalling pathways, by blocking the phosphorylation of p65 RelA subunit specifically at serine-536. This action prevents p65's activation by stopping its translocation into the nucleus. BTK inhibition in multiple myeloma cells led to the down-regulation of anti-apoptotic proteins Bcl-xL, FLIPL and survivin, and increased caspase-mediated apoptotic death within the malignant plasma cells. These data provide a platform for the clinical trialling of ibrutinib (and other BTK inhibitors) in the treatments of human multiple myeloma. In particular, this work shows there is a strong rationale for the use of ibrutinib in combination therapy with the current multiple myeloma chemotherapeutic bortezomib.