1-Benzy1-2-phenylbenzimidazole, a benzimidazole derivative, triggers chondrosarcoma cell apoptosis via caspase-dependent and independent pathways

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ABSTRACT INTRODUCTION:

Chondrosarcomas are malignant tumours showing cartilage differentiation, and it is the third of most common primary bone malignancy after myeloma and osteosarcoma, accounting for approximately 20% of bone sarcomas and mainly affecting the middle-aged population. The mostly chondrosarcomas are slow growing and rarely metastasize. Due to its resistance to both ionizing radiation and chemotherapy, chondrosarcoma is making the management of chondrosarcoma a complicated challenge. Clinically, surgical resection remains the primary mode of therapy for chondrosarcoma. In the absence of an effective adjuvant therapy, this mesenchymal malignancy has a poor prognosis and novel and adequate therapies are needed. Benzimidazole derivatives have a wide range of biological and pharmacological activities with therapeutic potential such as antihistamine, antiulcerative, anti-inflammatory, antioxidant, anti-HIV-1, antibacterial and anticancer activities. However, the roles of benzimidazole derivatives in chondrosarcoma remain largely undefined. In this study, we synthesized a new benzimidazole derivative 1-benzyl-2-phenylbenzimidazole (BPB) and investigated its anticancer activity in human chondrosarcoma cells. Our data provide evidence in human chondrosarcoma cell, that BPB decreased cells survival and tumor growth both in vitro and in vivo.

METHODS:

Cell culture: The human chondrosarcoma cell lines (JJ012 and SW1353) and Primary cultures of human chondrocytes which were isolated from articular cartilage were cultured in DMEM/10-MEM which were supplemented with 10% fetal bovine serum (FBS) and maintained at 37 °C in a humidified atmosphere of 5% CO2. MTT assay, Colony assay, DAPI staining, Flow Cytometry, Western blot analysis, Caspase activity assay, Immuno fluorescent staining.

RESULTS SECTION:

To investigate the potential for BPB to induce cell death in human chondrosarcoma cells, we first examined the effect of BPB on cell survival in human chondrosarcoma cells using the MTT assay. Treatment of JJ012 and SW1353 cells with BPB induced cell death in a concentration-dependent manner (Fig. 1A) and increased the condensation of chromatin by DAPI staining using immunofluorescence microscopy (Fig. 1B). Treating cells with BPB increased the concentration-dependent increase in cell death, resulting in an increase in the percentage of cells in the sub G1 phase (Fig. 2A-B). Annexin V/ PI double -labeling was used to detect PS externalization. Compared to vehicle-treated cells, a high proportion of Annexin V labeling was detected in cells treated with BPB (Fig. 2C-D).

To determine whether BPB induced apoptosis by triggering the extrinsic apoptotic pathway, JJ012 cells were treated with BPB. Treatment with BPB induced an increase in Fas and FADD protein levels, and increased activity of the upstream protein caspase 8 in JJ012 cells (Fig. 3A-B). Treating JJ012 cells with BPB promoted Bax, Bak and Bad protein levels but decreased Bcl-2, Bcl-xL and Bid protein levels (Fig. 3C). We detect the mitochondrial membrane potential by using the mitochondria-sensitive dye JC-1 and flow cytomtery, as shown in Fig. 3D, and we found BPB-increased cell death is mediated by mitochondrial dysfunction.

The recent study shows that BPB induces a disruption of ΔΨm and causes the release of cytochrome c, AIF and Endo G into the cytoplasm (Fig. 4A). We found that BPB promotes the protein levels of AIF and Endo G in the nuclei by immunofluorescent and Western blot analysis (Fig. 4A-B). The results also show that siRNA transfection inhibited the expression of AIF and Endo G, and blocked BPB-induced cell death (Fig. 4C). These observations demonstrate that BPB induces apoptosis through the mitochondrial dependent pathway.

DISCUSSION:

The diagnosis of chondrosarcoma is easily ignored because of its infrequent occurrence, its indolent pattern of growth, and the difficulty in identifying it from chondroma in histopathology. We found a new benzimidazole derivative BPB induced cell death in human chondrosarcoma cell lines but not in primary chondrocytes. Our data indicate that the novel benzimidazole derivative BPB induces cell death in human chondrosarcoma cells both in vitro and in vivo. The flow chart shows that BPB triggers apoptosis by regulating Fas, FADD, caspase 8, bid, Bax and Bcl-2 and through activation of the caspase cascade (caspase-3, -8 and -9) or dysfunction of mitochondria which releases AIF and Endo G then causing apoptosis. These results provide a potential molecular mechanism for BPB-induced apoptosis in chondrosarcoma cells. Thus, BPB is a promising chemotherapeutic agent worthy of further development for treatment of human chondrosarcoma cells.