Role of the Transient Receptor Potential Vanilloid-type2 Agonist Cannabidiol in Multiple Mieloma

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Background: Multiple myeloma (MM) is a B-cell malignancy characterized by an accumulation of a monoclonal CD138+ plasma cell (PCs) populations. Multistep transformation processes occur as activation of the NF- κB, RAS/RAF/MEK/MAPK/ERK and PI3K/AKT pathways that promote proliferation and prevent MM cell death [1]. Transient Receptor Potential Vanilloid-type2 (TRPV2) is a cation channel, located in 17p11.2, involved in cancer growth and progression [2]. Cannabidiol (CBD) is TRPV2 agonist, currently investigated for its potential therapeutic application in the management of cancer. CBD activation of TRPV2 in glioma cell lines results in decreased proliferation and increased susceptibility to drug-induced cell death [2]. No findings were available on TRPV2 in MM cells. The aim of the present study was to determine the role of TRPV2 and CBD in MM. Methods: CD138 and TRPV2 were evaluated by cytofluorimetric analysis using anti-CD138-PE, goat anti-TRPV2 and donkey anti-goat-FITC antibodies in PCs derived from 13 firstly diagnosed MM patients and in RPMI8226 MM cell line. $CD138^+$ RPMI8226 cells were transiently transfected with TRPV2 gene [2]. Untransfected and TRPV2-transfected MM cell line were treated up to 3 days with CBD 1-30 micromolar (stock solution 25 mM in DMSO) alone or in combination with antagonists for CB1 (AM251, 25 micromolar from stock solution 25 mM in DMSO), CB2 (AM630, 20 micromolar from stock solution 25 mM in DMSO), TRP (Ruthenium Red, 10 micromolar from stock solution 50 mM in H_2O) receptors, the viability was evaluated by MTT assay [2]. The CBD dose of 20 micromolar was choose as lower effective dose. CBD actions were determined in proliferation using BrdU Cell Proliferation Assay (Millipore), and in modulating cell cycle by PI staining (20 micromolar). Cell death was evaluated by DNA fragmentation assay (20 micromolar PI staining) and agarose gel analysis. NF-κB DNA binding activity, was quantified in nuclear extract using TransAm Flexi NF-kB ELISA assay (Active Motif). The statistical significance of results was determined by ANOVA or student's t-test, *p<0.01Results: We demonstrated the presence of heterogeneous $CD138^+TRPV2^+$ and $CD138^+TRPV2^-$ PC populations in MM patient and only $CD138^+$ $TRPV2^-$ cells were found in MM cell line. CBD reduced in a TRPV2-dependent and independent manner survival and proliferation of MM cell lines. In fact, 20uM CBD was able to decrease cell viability and proliferation in $CD138^+TRPV2^+$ respect to $CD138^+TRPV2^-$ RPMI (69% vs. 52% cell viability; 48% vs. 67% cell proliferation). Moreover, CBD induced cell cycle arrest with an increase in the G0/G1 phase with major effects in $CD138^+TRPV2^+$ (57%) respect to $CD138^+TRPV2^-$ (45%). CBD-induced cell death was demonstrated by DNA fragmentation with a major effect in $CD138^+TRPV2^+$ cells. In addition, CBD negatively regulated p52/relB, more in $CD138^+TRPV2^+$ respect to $CD138^+TRPV2^-$ cells (43% vs. 57%). Moreover, only in $CD138^+TRPV2^+$ cells there was a reduction of p65 expression compare to untreated cells (39%).Conclusion: CBD arrests cell cycle at G0/G1 phase and induces necrotic cell death in MM cell lines, with the higher effect was evidenced on $CD138^+TRPV2^+$

cells. [1] Rajkumar SV, Am J Hematol. 86:57, 2011 [2] Nabissi M et al, Carcinogenesis 34:48, 2013