Salinomycin As An Apoptosis Regulator Of Osteosarcoma Cell Line U2OS Through The Intrinsic Pathway By Regulating The Expression Of Bax And Bcl2

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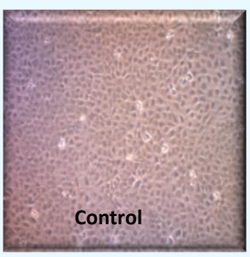
Background *:* Osteosarcoma poses a serious therapeutic challenge, mainly due to heterogeneous nature of the cells, which is aggressive and progressive. Doxorubicin has shown efficacy as a therapeutic agent in osteosarcoma, but in the process of therapy doxorubicin often experiences resistance caused by several mechanisms. Until now there has been no significant improvement in the prognosis of osteosarcoma in four decades. Several drugs have been studied for their anti-cancer effects. Salinomycin is believed to have anti-cancer effects against a variety of malignancies.

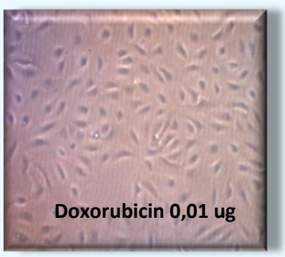
Obejective : Comparing the effects of salinomycin with doxorubicin as an apoptosis regulator by assessing Bcl2 and Bax gene expression in U2OS osteosarcoma cell cultures

Cell culture and drug sensitivity assay : U2OS cells line (ATCC) cultured in Dulbecco's modified Eagle medium with 10% *fetal bovine serum* (Invitrogen, Carlsbad, CA). Then cells in the subculture. Once the cell reaches confluence, several concentrations of salinomycin and doxorubicin are added to determine the dose of cytotoxin and analyzed

qRT-PCR assay : U2OS cells were grown in *6 wells tissue cultures* and added 0.1 µg doxorubicin, 0.005 M salinomycin; 0.0125 M, µand 0.025 Mµ. Then the cells were incubated for 48 hours, a population of living cells extracted to obtain mRNA.All target genes are normalized to GAPDH/*beta actin* and expressed in *fold change*. Relative gene expression is calculated by the $2^{-\Delta\Delta C_T}$ (Livak & Schmittgen 2001).

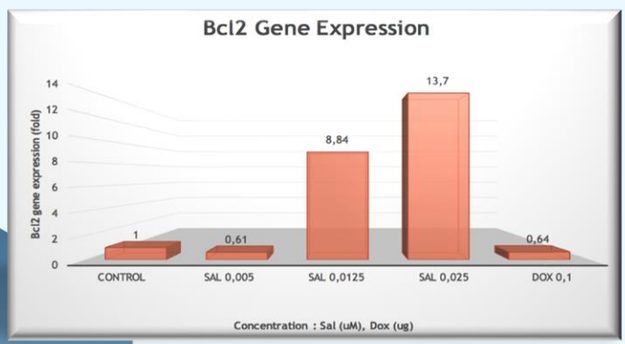
GADPH foward : CGG ATT GG TCG TAT TGG, GADPH reverse : TCA AGG TGT GAG GAC TGG, Bax forward : CCC GAG AGG TCT TTT TCC GAG, Bax reverse : CCA GCC CAT GAT GGT TCT GAT, Bcl2 forward : GCT CTA AA TCC ATC CAG, Bcl2 reverse : CCT CTC CAT CAT CAA CTT

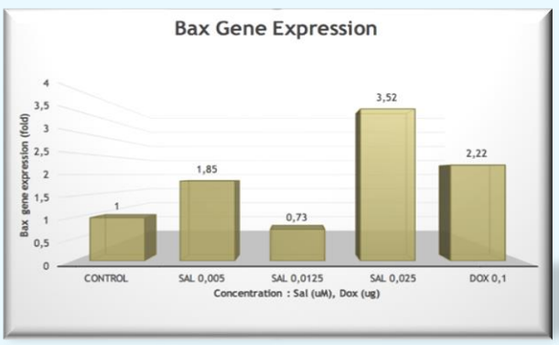




Statistical analysis : The results are expressed as the means ± standard deviation (SD). The Anova test was used for the statistical analyses, and P<0.05 was considered to be significant.

Result : This study reported that Bax expression at a salinomycin concentration of 0.005 µM was 1,85 ± 0,167 -fold higher compared to control cells (p= 0,002), while at the same concentration, Bcl2 expression was found to be 0,61 ± 0,57 -fold lower when compared to control cells (p<0,001).





Conclusion : This underscores the potential of salinomycin as a standalone anti-apoptotic pathway modulator in the management of osteosarcoma. Consequently, this investigation not only contributes to our comprehension of these therapeutic agents but also situates their collective impact on apoptosis regulators, providing valuable insights for the refinement of osteosarcoma treatment paradigms

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