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Synergistic effect of ascorbate in napthoquinone induced cytotoxicity, ROS/RNS generation and inhibition of metastasis in vitro

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Juglone (5-hydroxy-1, 4-Napthoquinone), and structural analogue Plumbagin (5-hydroxy-2-methyl-1, 4-naphthoquinone) are natural product pigments. As per previous studies in our lab it was established that these compounds modulates inflammatory cytokines in RAW264.7 and THP-1 cell lines while significantly regressed C57BL/6-derived B16 melanoma. With an objective to investigate the mechanism of action of these napthoquinones in cytotoxicity and ROS generation in ARO (undifferentiated/ anaplastic thyroid cancer cell line); MTT and DCFDA fluorescent dye assays were performed respectively. Directional cell migration in vitro was studied by wound healing assay while colony formation in ARO cells in response to napthoquinones was studied by clonogenic assay. IC50 values of Plumbagin and Juglone in ARO cell line was found to be around 8-10 μM and 12-15 μM respectively. This cytotoxicity was restored by antioxidants NAC and GSH while increased by ascorbic acid. Cellular ROS/RNS was generated by Plumbagin and Juglone in ARO cell line. It was observed that nitric oxide (NO) inhibitors Aminoguanidine (AG) and NG-nitro-l-arginine methyl ester (L-NAME) in presence of Plumbagin (10 uM) and Juglone (15 μM) increases cellular ROS/RNS with respect to LNAME and AG controls. Ability of ARO cells to migrate and replenish the wound and form colony was significantly reduced with ascorbate and compounds together than with compound alone. ARO cells did not form colony above 2.5 μM concentration. Preliminary results shows ascorbate in presence of napthoquinone synergizes cytoxicity, wound healing and colony formation (metastasis markers) in ARO cell line. Antioxidants and NO inhibitors modulate direct action of napthoquinones in ARO. Role of Ascorbate in cellular pathways is under study.

Biography

Sujay Gaikwad is working with Government of India, Department of Atomic Energy, and Bhabha Atomic Research Centre as Scientific Officer D.

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