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Non-Steroidal Microtubule Disrupting Analogues Induce Programmed Cell Death in Breast and Lung Cancer Cell Lines

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Abstract : A tetrahydroisoquinolinone (THIQ) core can be used to mimic the A,B-ring of colchicine site-binding microtubule disruptors such as 2-methoxyestradiol in the design of anti-cancer agents. Steroidomimeric microtubule disruptors were synthesized by introducing C'2 and C'3 of the steroidal A-ring to C'6 and C'7 of the THIQ core and by introducing a decorated hydrogen bond acceptor motif projecting from the steroidal D-ring to N'2. For this in vitro study, four non-steroidal THIQ-based analogues were investigated and comparative studies were done between the non-sulphamoylated compound STX 3450 and the sulphamoylated compounds STX 2895, STX 3329 and STX 3451. The objective of this study was to investigate the modes of cell death induced by these four THIQ-based analogues in A549 lung carcinoma epithelial cells and metastatic breast adenocarcinoma MDA-MB-231 cells. Cytotoxicity studies to determine the half maximal growth inhibitory concentrations were done using spectrophotometric quantification via crystal violet staining and lactate dehydrogenase (LDH) assays. Microtubule integrity and morphologic changes of exposed cells were investigated using polarization-optical transmitted light differential interference contrast microscopy, transmission electron microscopy and confocal microscopy. Flow cytometric quantification was used to determine apoptosis induction and the effect that THIQ-based analogues have on cell cycle progression. Signal transduction pathways were elucidated by quantification of the mitochondrial membrane integrity, cytochrome c release and caspase 3, -6 and -8 activation. Induction of autophagic cell death by the THIQ-based analogues was investigated by morphological assessment of fluorescent monodansylcadaverine (MDC) staining of acidic vacuoles and by quantifying aggresome formation via flow cytometry. Results revealed that these non-steroidal microtubule disrupting analogues inhibited 50% of cell growth at nanomolar concentrations. Immunofluorescence microscopy indicated microtubule depolarization and the resultant mitotic arrest was further confirmed through cell cycle analysis. Apoptosis induction via the intrinsic pathway was observed due to depolarization of the mitochondrial membrane, induction of cytochrome c release as well as, caspase 3 activation. Potential involvement of programmed cell death type II was observed due to the presence of acidic vacuoles and aggresome formation. Necrotic cell death did not contribute significantly, indicated by stable LDH levels. This in vitro study revealed the induction of the intrinsic apoptotic pathway as well as possible involvement of autophagy after exposure to these THIQ-based analogues in both MDA-MB-231- and A549 cells. Further investigation of this series of anticancer drugs still needs to be conducted to elucidate the temporal, mechanistic and functional crosstalk mechanisms between the two observed programmed cell deaths pathways.

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