EVALUATION OF SYNTHETIC "TUBULYSIN U" INDUCED CYTOTOXICITY IN HL60, C6, PC12 AND ECV304 CELL LINES

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Microtubules are small tubes composed by tubulin found throughout the cytoplasm of eukaryotic cells. They are involved in basic cellular processes as maintenance of cell shape, extracellular and intracellular transport, movement of cells and mitosis, so their irreversible elimination causes cell death. Since uncontrolled cell proliferation is one of the main features of cancer, drugs which interfere with microtubule thereby interrupting cell division are considered helpful anticancer tools. Agents which inhibit microtubule polymerization, such as the vinca alkaloid, colchicines and dolastatins are commonly used in clinical practice. These drugs are effective but they often induce drug resistance, hence the demand of new compounds with a more specific profile and/or improved efficacy. During a screening of Mixobacteria for bioactive natural compounds, F. Sasse et al. discovered tubulysins, antimitotic compounds that rapidly degrade the tubulin cytoskeleton displaying antimitotic properties in cultured cells. Ongoing research is addressed to alter the chemical structure of natural tubulysins in order to obtain synthetic molecules which may be used for cancer treatment.

According to our present study, the synthetically challenging tubulysin structural framework is now accessible through a reliable and modular reaction sequence allowing for the preparation of hundreds of milligrams of the stereochemically pure tetrapeptides tubulysins U and V. Preliminary cytotoxicity studies have been carried out using tubulysin U. Antiproliferative effect has been evaluated in cancer cell lines HL60 (human leukaemia), C6 (rat glioma) and PC12 (rat pheochromocytoma). In addition, since angiogenesis is an important feature of tumor growth, we tested tubulysin U on the human endothelial ECV304 cell line. Cells were exposed to different concentrations ($10^{-11} \div 10^{-4}$ M) of tubulysin U for 24, 48 and 72 hours.

The ATPlite ATP monitoring system revealed that in HL60 cell line synthetic tubulysin U induced cell death at nanomolar concentrations in a dose- and time-dependent manner with EC_{50} values of 4.41×10^{-7} M, 1.08×10^{-8} M and 4.98×10^{-9} M at 24, 48 and 72 hours, respectively. Moreover, tubulysin U exerted an antiproliferative activity in C6, PC12 and ECV304 cells, although with less potency and with EC50 values reaching the plateau within 48 hours.

The present study confirms the antimitotic properties of tubulysin class and provides experimental evidence that among this class different compounds may be useful pharmacological tools as novel anticancer agents.