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Antitumor signalling of a novel microtubule inhibitor in human epithelial cervical carcinoma

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INTRODUCTION:
Cervical cancer is one the world's most common female carcinomas with majority of international cases arising in developing countries. Although the initiation of the human papillomavirus vaccine may change the disease epidemiology over the coming decades, improvement in the treatment of this malignancy is still tenable. Recent chemotherapeutics aim to target the cell cycle triggering arrest and eventual programmed cell death. A novel *in silico*-designed sulphamoylated estradiol analogue, namely 2-ethyl-3-O-sulphamoyl-estra-1,3,5(10)16-tetraene (ESE-16), was designed by our laboratory and synthesised to be explored for its anti-cancer potential. Previous studies revealed *in vitro* antiproliferative effects of ESE-16 on various carcinoma cell lines and the induction of cell death after 24h of exposure.

AIM:
This *in vitro* study investigated the influence of ESE-16 on morphology, cell cycle and the generation of reactive oxygen species (ROS) in cervical carcinoma cells (HeLa cells), in order to elucidate the mechanism/s of cell death.

MATERIALS AND METHODS:
The 50% growth inhibitory concentration was determined, using real-time cell analyses and spectrophotometry, to be 0.2µM after 24h of exposure (data not reported). Cell morphology observations were performed by means of light- (PlasDIC, and haematoxylin and eosin staining) and transmission electron-microscopy (TEM). Flow cytometry using the fluorescent activated cell sorting FC500 system was employed to determine the compound's effect on the cycle of the HeLa cells, induction of ROS and the initiation of programmed cell death.

RESULTS:
Microscopy techniques revealed cells blocked in metaphase and characteristics of apoptosis after 24h of exposure to ESE-16. Furthermore TEM also demonstrated the presence of autophagic vesicles suggesting the presence of autophagy. Flow cytometric studies showed that ESE-16 causes (i) specific Annexin-V antibody staining; an increase in both (ii) apoptotic cells (sub-G1) in cell cycle and (iii) ROS production after 24h, as well as upregulation of the autophagy-gene 5 (Atg5), thereby suggesting the simultaneous induction of apoptosis and autophagy. Spectrophotometry also established amplification in caspase 3 activity after treatment of HeLa cells to ESE-16.

CONCLUSION:
Data from this study revealed that ESE-16 has the ability to induce both apoptotic and autophagic cell death processes. These are the preferred means of cell death in this environment, since these programmed pathways do not illicit an immune response. Future *in vitro* studies are warranted to unravel the common signalling molecules involved in the crosstalk action mechanism between these two types of cell death, potentially providing further understanding for *in vivo* studies on this potential, novel anti-cancer agent.