INFLUENCE OF 2-METHOXYESTRADIOL-BIS-SULFAMATE ON CELL GROWTH, MORPHOLOGY AND CELL DEATH IN MCF-7 AND MCF-12A CELLS

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Abstract

2-methoxyestradiol (2ME2) is an endogenous estrogen metabolite that has antiproliferative activity, antiangiogenic activity and anti-tumor activity. 2ME2 has shown positive results as a potential treatment of many types of cancer, in particular breast cancer. Inhibition of proliferation results mainly from the induction of apoptosis as it appears that 2ME2 targets active proliferating cells and thus quiescent cells are not affected. Several promising analogues of 2ME2 have been developed in recent years. 2-methoxyestradiol-bis-sulfamate (2-MeOE2bisMATE) is a novel possible anti-cancer agent currently not commercially available with the potential of improved bioavailability and potency when compared to 2ME2. 2-MeOE2bisMATE is a derivative of oestrene-3,17β-sulfamate (EMATE) and can be regarded as a potent inhibitor of steroid sulfatase (STS) activity. STS takes part in hydrolysis of oestrone sulfate to oestrone. This possibly contributes to the oestradiol production in breast tumor tissues. Although 2-MeOE2bisMATE holds enormous therapeutic promise, the signal transduction pathway needs to be unravelled. Thus, this project will add to the knowledge regarding the elucidating molecular mechanisms of the analogue in order to contribute to the design/improvement of 2ME2 analogues with therapeutic potential.

Introduction

The aim of this practical in vitro study was to investigate the influence 2-MeOE2bisMATE on cell growth, morphology and cell death in a breast cancer cell line (MCF-7) and a non-tumorigenic epithelial breast cell line (MCF-12A) respectively.

Materials & Methods

- 2-MeOE2bisMATE: was synthesized by Professor Visagie from the Department of Chemistry (University of Pretoria, South Africa), since the compound is not commercially available.
- MCF-7: This tumorigenic adherent breast epithelial cell line was commercially available from HighVeld Biological (Pty) Ltd. (Pretoria, South Africa).
- MCF-12A: This non-tumorigenic adherent human breast epithelial cell line was a gift from Professor Parker (Department of Medical Biochemistry, University of Cape Town, South Africa).

Crystal violet staining: was used to investigate the effects on cell numbers by conducting time- and dose-dependent studies.

Light microscopy (phase-contrast & DAPI stain): was used to visualise the effects of 2-MeOE2bisMATE on cell morphology.

Fluorescence microscopy (Propidium iodide fluorescent microscopy): was used to visualise apoptosis (nucleosomal and caspase dependent) and autophagy (lysosomes and vacuoles).

Transmission electron microscopy (TEM): was used to investigate effects on the internal cell structure.

Results / Discussion

Determination of cell numbers

The effects of a 2-MeOE2bisMATE (0.2 μM) at different time intervals (24 hours, 36 hours, 48 hours and 72 hours) were evaluated in MCF-7 and MCF-12A cell lines. Exposure of 48 hours resulted in 47% growth reduction in MCF-7 cells and 79% in MCF-12A cell lines after 24 hours of exposure. Exposure of 48 hours resulted in 47% growth reduction in MCF-7 cells and 79% in MCF-12A cells while 72 hour exposure resulted in 32% growth reduction in MCF-7 and 78% in MCF-12A cells respectively. (data not shown). In addition, findings also indicate that 2-MeOE2bisMATE decreases growth in a time- and dose-dependent manner. These spectrophotometric results suggest that 2ME2 cells are more susceptible to MCF-12A cells.

Fluorescence microscopy

Propidium iodide staining was used to obtain a quantitative analysis of cell proliferation, the effects on cell numbers by conducting time- and dose-dependent studies. The effects of 2-MeOE2bisMATE-treated MCF-7 and MCF-12A cells line using crystal violet staining (Figure 1a and Figure 2a). 0.4 μM 2-MeOE2bisMATE decreased cell numbers to 75% in MCF-7 cells in contrast to 52% in MCF-12A cells after 24 hours of exposure. Exposure of 48 hours resulted in 47% growth reduction in MCF-7 cells and 79% in MCF-12A cells while 72 hour exposure resulted in 32% growth reduction in MCF-7 and 78% in MCF-12A cells respectively. (data not shown). In addition, findings also indicate that 2-MeOE2bisMATE decreases cell growth in a time- and dose-dependent manner. These spectrophotometric results suggest that 2ME2 cells are more susceptible to MCF-12A cells.

Transmission electron microscopy

TEM was conducted to visualize the in vitro effects on morphology after 48 hour exposure to 2-MeOE2bisMATE. Mitochondria blebbing and chromatin condensation were observed in both cell lines (MCF-7) (Figure 3a) and MCF-12A (Figure 4). However, at 48 hours intracellular vacuoles were observed in MCF-7 cells only. Occurrence of promyelocyte-like vacuoles indicates that apoptosis and autophagy are involved. In addition, the effects observed in MCF-7 cells were more prominent and severe than the effects in the MCF-12A cells.

Light microscopy

96 hour treated cells (in 5 X 104 to 105/mL) were used to determine the influence of 2-MeOE2bisMATE on the cell morphology. Cells were treated with 0.4 μM 2-MeOE2bisMATE for 48 hours. Treated MCF-7 cells (Figure 5) revealed hypercondensed chromatin, apoptotic blebs and a mitotic block was not observed. In addition, MCF-12A cells (Figure 6) revealed various apoptotic tendencies; however, it was less prominent.

Conclusion

This preliminary study indicates that 2-MeOE2bisMATE may be a differential action mechanism in non-tumorigenic cells (MCF-12A) when compared to tumorigenic cells (MCF-7). This provides appropriate basis for future research in the investigation of 2-MeOE2bisMATE in tumorigenic cells and non-tumorigenic cells in order to determine the chemical signalling pathway and action mechanism exerted by 2-MeOE2bisMATE in tumorigenic and non-tumorigenic cells.

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References