

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

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## Introduction

2-methoxyestradiol (2ME2) is an endogenous estradiol metabolite that has antiproliferative activity, antiangiogenic activity and antitumor 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. However, 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 oestrone-3-O-sulphamate (EMATE) and can be regarded as a potent inhibitor of steroid sulphatase (STS) activity. STS takes part in hydrolysis of oestrone sulphate 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 unraveled. 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.

## Aim

The aim of this preclinical *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 Vleggar 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. (Sandringham, 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 (Haematoxylin & Eosin stain):** was used to visualize the effects of 2-MeOE2bisMATE on cell morphology.
- Fluorescence microscopy (Propidium iodide / Hoechst 33342 / Acridine orange):** was used to visualize autophagy (lysosomes) and apoptosis.
- Transmission electron microscopy (TEM):** was used to investigate effects on the internal cell structure.

## Results / Discussion

### Determination of cell numbers

To obtain a quantitative analysis of cell proliferation, the effects of a 2-MeOE2bisMATE (0.2µM, 0.4 µM, 0.6 µM, 0.8 µM and 1 µM) at different time intervals (24 hours, 36 hours, 48 hours and 72 hours) were evaluated in the MCF-7 and MCF-12A cell line using crystal violet staining (Figure 1 and Figure 2). 0.4µM 2-MeOE2bisMATE decreased cell numbers to 75% in MCF-7 cells in contrast to 92% 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 33% growth reduction in MCF-7 and 78% in MCF-12A respectively (data not shown). In addition, findings also indicate that 2-MeOE2bisMATE decreases cell growth in a time- and dose-dependent manner. These spectrophotometrical results suggest that MCF-7 cells are more susceptible than MCF-12A cells.

### Transmission electron microscopy

TEM was conducted to investigate the *in vitro* effects on morphology after 48 hour exposure to 0.4µM 2-MeOE2bisMATE. Membrane blebbing and chromatin condensation were observed in both cell lines (MCF-7 (Figure 3) and MCF-12A (Figure 4)). However, at 48 hours intracellular vacuoles were observed in MCF-7 cells only. Occurrence of membrane blebbing and 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.

### Fluorescence microscopy

Hoechst 33342 was used to stain the DNA and propidium iodide (PI) was used as a probe to detect if the membrane was compromised. This is a triple staining method. Acridine orange is lysosomotropic fluorescent compound that serves as a tracer for acidic vesicular organelles including autophagic vacuoles and lysosomes. A 48 hour exposure of 0.4µM 2-MeOE2bisMATE in MCF-7 (Figure 5) and MCF-12 (Figure 6) cell lines revealed increased presence of autophagy when compared to the controls. However, autophagy was more prominent in MCF-7 cells than in MCF-12A cells.

### Light microscopy

Haematoxylin and eosin (H & E) staining was used to determine the influence of 2-MeOE2bisMATE on the cell morphology. Cells were treated with 0.4µM 2-MeOE2bisMATE for 48 hours. Treated MCF-7 cells (Figure 7) revealed hypercondensed chromatin, apoptotic bodies and a mitotic block was observed. In addition, MCF-12A cells (Figure 8) revealed various apoptotic tendencies, however, it was less prominent.

## Light microscopy

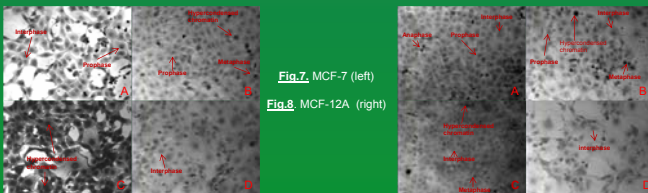


Fig.7, MCF-7 (left)  
Fig.8, MCF-12A (right)

Figure 7 and figure 8. Cells were grown on sterile coverslips and exposed for 48 hours; subsequently cells were stained with haematoxylin (nucleus) and eosin (cytoplasm) and microphotographs were taken. MCF-7-treated cells revealed severe hypercondensed chromatin in contrast to MCF-12A-treated cells that does indicate hypercondensed chromatin however, less severe.

- Cells in culture medium;
- Vehicle-treated (DMSO) control;
- 0.4µM 2-MeOE2bisMATE-treated
- Starvation (autophagy)

## Conclusion

This preliminary study indicates that 2-MeOE2bisMATE exerts a differential action mechanism in non-tumorigenic cells (MCF-12A) when compared to tumorigenic cells (MCF-7). This provides appropriate basis for future research for the investigation of 2-MeOE2bisMATE as a possible anti-cancer agent. However, several questions still remain about the signaling pathway and action mechanism exerted by 2-MeOE2bisMATE in tumorigenic and non-tumorigenic cells.

## Acknowledgements

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## References

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## Cell numbers

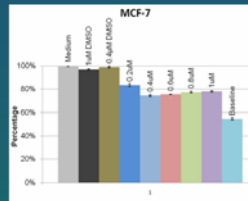


Figure 1a

Dose- and time-dependent studies using spectrophotometry were conducted.

24 Hour exposure

Fig.1a, MCF-7

Fig.1b, MCF-12A

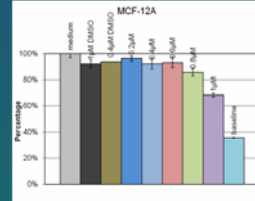


Figure 1b

Figure 1: 24 Hour 2-MeOE2bisMATE exposure of MCF-7 (left) and MCF-12A (right) resulted in significant growth inhibition. In the MCF-7 cell line however, growth inhibition in the MCF-12A cell line was not as severe. 0.4 µM 2-MeOE2bisMATE treatment decreased cell numbers to 75% in MCF-7 cells in contrast to 92% in MCF-12A cells after 24 hours of exposure.

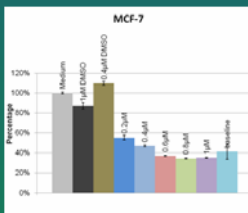


Figure 2a

48 Hour exposure

Fig.2a, MCF-7

Fig.2b, MCF-12A

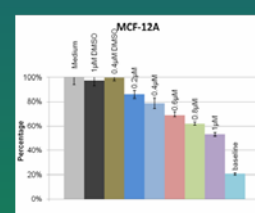
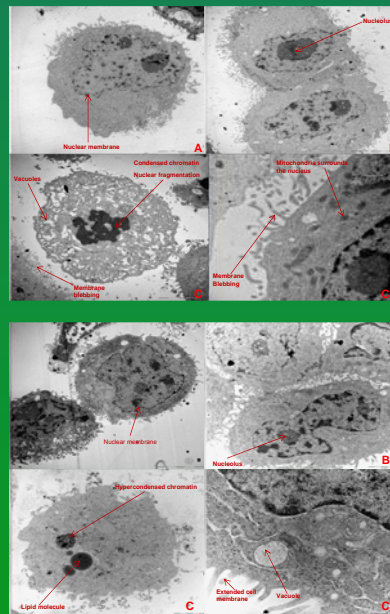


Figure 2b

Figure 2: 48 Hour 2-MeOE2bisMATE exposure in a MCF-7 cell line (left) and MCF-12A cell line (right) resulted in greater growth inhibition in the MCF-7 cell line. Exposure of 48 hours resulted in 47% growth reduction in MCF-7 and 79% in MCF-12A cell numbers.

## Transmission electron microscopy



Micrographs were taken at magnifications of 6000X, 10 000X, 200 00X and 500 000X.

Fig.3, MCF-7 (top)  
Fig.4, MCF-12A (bottom)

Figure 3: An 48 hour exposure in a MCF-7 cell line resulted in severe vacuole formation which implies the presence of autophagy. Hypercondensed chromatin and membrane blebbing occurrence indicate that apoptosis does indeed occur.

Figure 4: 48 Hour exposure in a MCF-12A cell line resulted in severe vacuole formation, hypercondensed chromatin and apoptotic body formation, this implies apoptosis as a form of cell death. Vacuole formation indicates the presence of autophagy. Apoptotic- and autophagic cell death tendencies are less prominent in the MCF-12A than the MCF-7 cell line.

- Cells in culture medium;
- Vehicle-treated (DMSO) control;
- 0.4µM 2-MeOE2bisMATE-treated

## Fluorescent microscopy

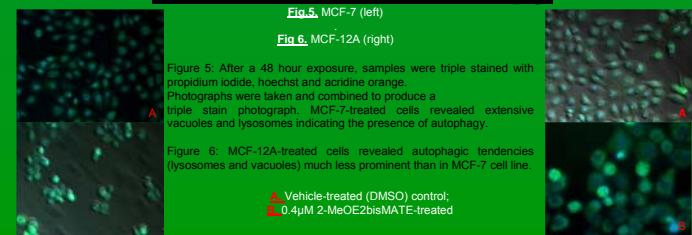


Fig.5, MCF-7 (left)

Fig.6, MCF-12A (right)

Figure 5: After a 48 hour exposure, samples were triple stained with propidium iodide, hoechst and acridine orange. Photographs were taken and combined to produce a triple stain photograph. MCF-7-treated cells revealed extensive vacuoles and lysosomes indicating the presence of autophagy.

Figure 6: MCF-12A-treated cells revealed autophagic tendencies (lysosomes and vacuoles) much less prominent than in MCF-7 cell line.

- Vehicle-treated (DMSO) control;
- 0.4µM 2-MeOE2bisMATE-treated