THE EFFECTS OF BIOACTIVE COMPOUNDS FROM THE MARINE MOLLUSC *Dicathais orbita* ON HUMAN REPRODUCTIVE CELLS AND HUMAN REPRODUCTIVE CANCER CELLS

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By V. Edwards
Bachelor of Biotechnology (Honours)

Department of Medical Biotechnology
School of Medicine
Faculty of Health Sciences
Flinders University
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ABSTRACT

Marine compounds which constitute the ancient purple dye, Tyrian purple have gained specific interest over the past few years for their biological specificity and cytotoxicity towards human cancer cells in comparison to primary cells. In particular, the indole-based compounds, 6-bromoisatin and tyrindoleninone from the Australia whelk, *Dicathais orbita*, are compounds of interest because of their anti-cancer activity and ability to induce apoptosis in several cancer cell lines, both *in vitro* and *in vivo*. Interestingly, the secretions from muricid marine molluscs are the source of a natural homeopathic remedy, 'Murex purpurea', sold for the treatment of a range of gynaecological disorders, including cancer of the uterus. However, to date the effects of these indole-based compounds on human reproductive cells is unknown. The objective of this project was to examine the effects of these natural indole compounds derived from *D. orbita*, on primary-derived female human granulosa cells, along with a series of female human reproductive cancer cells. The hypothesis of this research was that the novel bioactive compounds from *D. orbita* would exert selective cytotoxicity towards the reproductive cancerous cells while having minimal, or no effect on the reproductive primary cells.

In vitro cell screening assays are beneficial for examining the immediate cytotoxicity of novel compounds however an added benefit of screening on reproductive cells is that the effects of these compounds on certain cell functions, such as hormone synthesis can also be examined. Furthermore, the manipulation of steroid hormone synthesis using human chorionic gonadotrophin (hCG) can be induced to mimic the *in vivo* response of these cells. Therefore, a second aim of this thesis was to determine the effects of the muricid compounds on hormone synthesis by female reproductive cells.

To address the aims of this research initially the *in vitro* cell culture conditions were optimised and characterised using the choriocarcinoma JAr cell line. Cell metabolic activity and cell viability were investigated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl)tetrazolium bromide (MTT), the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetazolium (MTS) and the crystal violet assays. Measurement techniques for hormone synthesis comparing the radioimmunoassay (RIA) and enzyme-linked immuno-sorbent (ELISA) assay were also examined, to establish the most reliable and reproducible assay. The optimised assays were then used to determine the effects of the bioactive compounds from *D. orbita* and several synthetic analogues of these indole-based compounds on human reproductive cells. Mechanisms of cell death were further investigated using apoptotic and necrotic assays along with examining changes in nuclear fragmentation and cell morphology, as key features of cell death mechanisms.

The optimised cell culture assay for the JAr cell line was a seeding density of 20,000 JAr cells per well and 2h cell adherence period, followed by incubation with0.5mg/ml MTT for 1h. The intra- and inter-assay coefficients of variation were 11.3% and 10.9% respectively. Human choronic gonadotrophin (1,000mIU/ml) significantly increased progesterone synthesis after 2h ($n=4$; $p< 0.05$) as determined by the RIA.

The synthetic compounds, indirubin and 6'6-dibromoindirubin, were not cytotoxic to the human choriocarcinoma JAr cell line, whereas, 5-bromoisatin (100µg/ml) significantly reduced cell viability by 50% (IC_{50}; 442µM) after 4h (n=3; $p<0.01$) and caused a significant reduction in JAr cell numbers at ≤ 100µg/ml for 6, 8 10 and 24h incubation ($p < 0.001$) as determined by the MTT assay. In a similar manner, indirubin had no effect on primary granulosa cell viability, whereas 5-bromoisatin significantly reduced cell numbers at concentrations greater than 10µg/ml. Both indirubin and 5-bromoisatin did, however, stimulate progesterone synthesis at low concentrations (0.01µg/ml) after 48h in granulosa cells derived from women with normal reproductive physiology. Indirubin and 5-bromoisatin (100µg/ml) did however inhibit estradiol synthesis by granulosa cells derived from women with abnormal reproductive physiology after 24h exposure. The results of this research therefore do not support the use of indirubin, 6'6-dibromoindirubin and 5-bromoisatin for the treatment of female reproductive cancers.

The semi-purified compounds tyrindoleninone and 6-bromoisatin, extracted from the muricid whelk, *D. orbita*, significantly decreased all three reproductive cancer cell lines, KGN, JAr and OVCAR-3 at a concentration at least 100-fold lower than in the primary reproductive cells although, hCG and cAMP afforded some protection against the cytotoxic effects of the compounds. Furthermore, this research confirmed that tyrindoleninone and 6-bromoisatin activated cell death in the KGN cancer cell line by apoptosis rather than necrosis. Progesterone secretion was either inhibited or not affected in primary granulosa cells from women with abnormal reproductive physiology when treated with these natural compounds both in the presence and absence of hCG. If these results are at all indicative of the *in vivo* response, the results of this study would not support the use of these compounds for women while

pregnant, or trying to conceive. Conversely though, the stimulatory effect on estradiol synthesis by primary granulosa cells (albeit only cells from women with normal reproductive physiology), along with the inhibitory and anti-proliferative effect on reproductive cancer cells, could potentially be advantageous for women during menopause as an alternative to hormone replacement therapy.

In conclusion, this study highlights the importance of *in vitro* cell culture assays for assessing the effects of both natural and synthetic compounds on reproductive cytotoxicity and hormone synthesis, providing essential data which can support current *in vivo* animal assays.

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