Interrogating gynaecological cancer cell metabolism at different oxygen tensions reveals simvastatin as metabolic regulator

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Background:

Around 200,000 new cases of gynaecological cancers are diagnosed in Europe every year. Potentially 75% of these cancers could benefit from improved treatment regimes.

Gynaecological cancer cells have an increased glycolysis rate and lactate concentration which have been suggested to predict increased likelihood of metastasis, resistance to therapy and reduced survival in patients. Lactate transport in cancer cells is carried out by members of the monocarboxylate transporter (MCT)family, notably MCT1/4.

Thus, we hypothesized that pharmacologic inhibition of MCTs could improve treatment outcome by reducing glycolytic potential of these tumour cells.

Methods:

Extracellular Flux Assay: Glycolytic profiling of gynaecological cancer cells performed using Seahorse XF96 Extracellular Flux Analyzer (Seahorse Bioscience). Cells were plated in culture media, let to adhere for two hours and treated with 10 uM simvastatin or left untreated for 24 hours. Glycolytic profiling of gynaecological cancer cells was performed according to manufacturer's protocol.

Real-time PCR: Used to determine the expression levels of Hif1 downstream genes (Glut-1, VEGF and CA-IX) in cells exposed to Air or Hypoxia (3%O2) for 24 hours using SYBR green technology. mRNA was extracted from cells which has been subjected to XF analysis. The data was normalized to house keeping gene RPL1.

Results:

- The metabolic profiles of endometrial and cervical cell lines differ from each other:
  - Ishikawa cells utilize glycolysis for energy production and has more glycolytic capacity than CaSki, SiHa and Hec1A cells (Fig.1b).
  - Ishikawa cells have increased glycolysis and glycolytic capacity under hypoxic conditions (3% O2) (Fig. 1c).
- Treatment of 10 µM SV for 24 hours significantly reduced glycolytic capacity and the reserve of Ishikawa cell lines in air and hypoxia (Fig. 2).
- Under 3% O_2 exposure for 24 hours, Hif1 and its downstream target genes CA-IX, GLUT1 and VEGF are upregulated (Fig. 3).

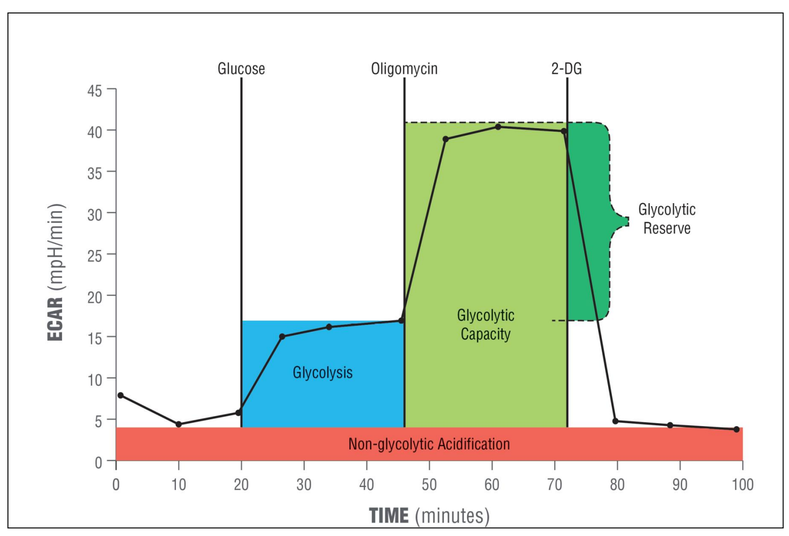

Figure 1a. Representative example of glycolytic function profile.

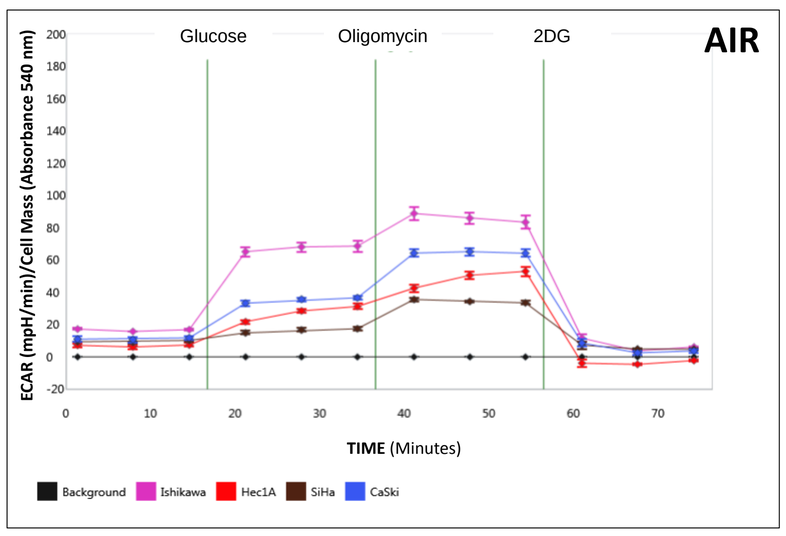

Figure 1b. Glycolytic profile of endometrial and cervical cell lines in air.

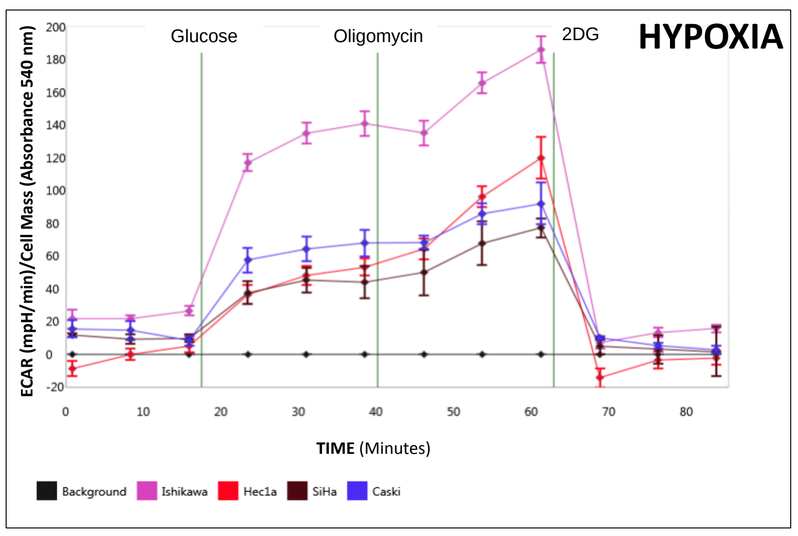

Figure 1c. Glycolytic profile of endometrial and cervical cell lines hypoxia .

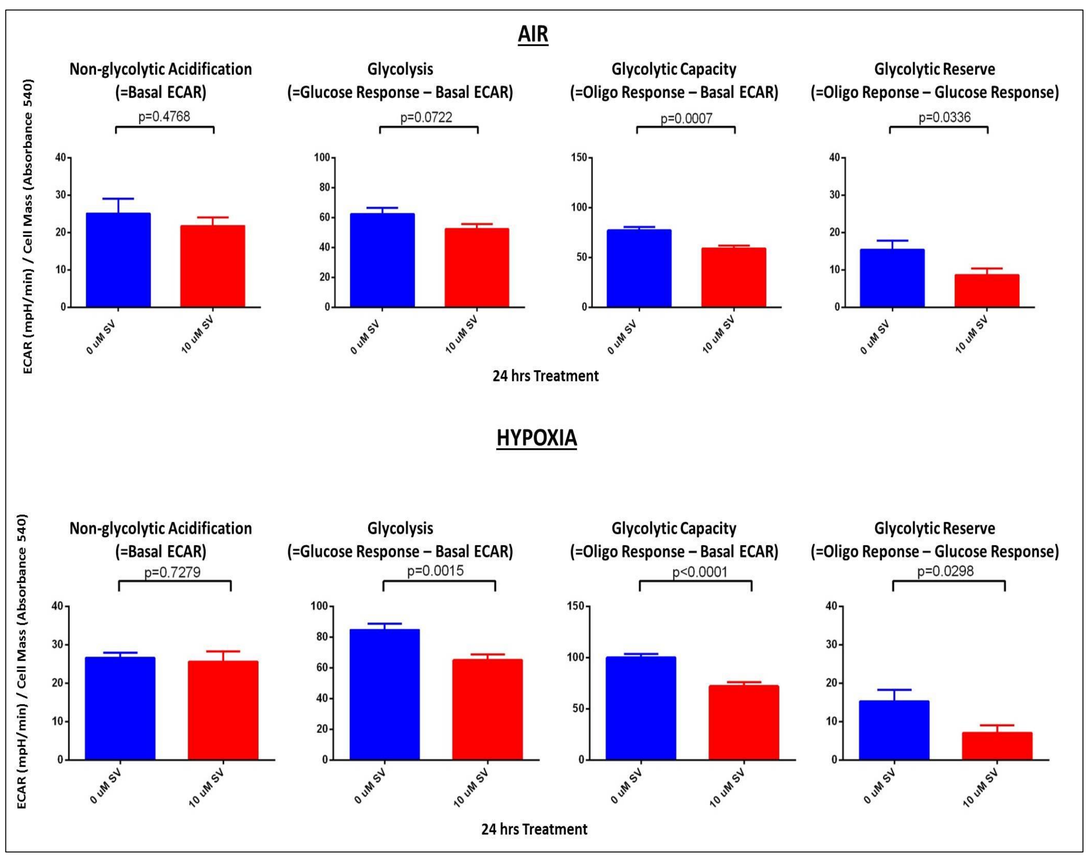

Figure 2. The effect of 24 hours 10 µM simvastatin treatment on ishikawa glycolysis in air and hypoxia (3%) (n=4/each).

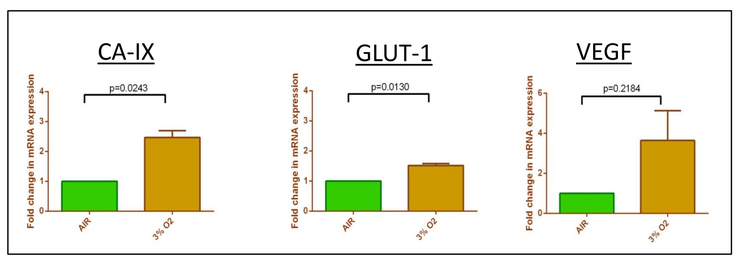

Figure 3. mRNA expression analysis of Hif1 regulated genes in air and hypoxia.(3% O2) (n=2). Expression of CA-IX, GLUT1 and VEGF increased relative to samples exposed to air.

Figure 4. Whitley i2 Instrument Workstation connected to a Whitley H35 HEPA Hypoxystation.

Conclusions:

Simvastatin;

- has significant impact on Ishikawa cellular metabolism - glycolysis.
- Thus, may be beneficial for the treatment of a variety of cancers.
- The Whitley i2 Workstation provides a controlled environment for reproducible measurement of hypoxic XF assays.

Future Directions:

To determine the lowest oxygen level in which cell metabolism can be successfully measured with a Seahorse XFe96 Analyzer.

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