Lack of the effect of low metformin concentrations on the response of breast cancer cell mitochondria to radiotherapy under conditions of the *in vitro* experimental hyperglycemia

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AGENDA

- BACKGROUND
- AIM AND SCIENTIFIC HYPOTHESIS
- RESULTS AND CONCLUSION
BACKGROUND

Cancer Diabetes

in vitro conditions
Organelles in focus

Who controls the ATP supply in cancer cells? Biochemistry lessons to understand cancer energy metabolism

Rafael Moreno-Sánchez a,*, Alvaro Marín-Hernández a, Emma Saavedra a, Juan P. Pardo b, Stephen J. Ralph c, Sara Rodríguez-Enríquez a, d

 Otto Warburg (1931)

cancer cells. Then, it follows that this aspect of the Warburg hypothesis is correct in the sense that most cancer cells have increased glycolytic flux, whereas there is no evidence that OxPhos is commonly impaired in cancer cells. As shown above, OxPhos can be the main cancer ATP supplier depending on cell growth conditions or feeding substrates. It is worth mentioning that 12–15 years ago studies on mitochondrial energy metabolism were difficult to publish and therefore few of them can be found in the literature.

tal assessment. Thus, in order to “demonstrate” that the Warburg hypothesis was a universal law, it has become acceptable to assume that respiratory rates, OxPhos and mitochondria are non-functional in cancer cells. This belief has become unfortunately a Central Dogma of Cancer Metabolism.
1957 - first application in anti-diabetic therapy (type 2 diabetes)

Web of knowledge

(1)
Number of publications on anticaner activity of metformin (ca. 1500)

(2)
Number of publications on metformin and radiation (ca. 170)
Number of publications on metformin, radiation and mitochondria (9)
Metformin enhances the response to radiotherapy in diabetic patients with rectal cancer

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IDEA OF OUR PROJECT

AIM

MITOCHONDRIA

THERAPY

METFORMIN + RADIATION

PATHOLOGY

DIABETES + CANCER
**EXPECTED EFFEKT:** reduction in cell viability inhibition in mitochondrial bioenergetics
Verification whether the mitochondria of breast cancer cells (4T1 cell line) could be an important target for agents such as: metformin (MET, the most widely used medication for type 2 diabetes) methylglyoxal (MG, a product of carbonyl stress in patients with diabetes) and X radiation (used in radiotherapy).

**HYPOTHESIS**

*Carbonyl stress combined with X rays increases anti-cancer properties of metformin via mitochondria under in vitro conditions*
EXPERIMENTAL CONDITIONS

Breast cancer cells (4T1 cell line)

Metformin at 1.5mM

Methylglyoxal at 50µM

X radiation at 2Gy/1min (1 min)
Bioenergetics experiments were carried out on digitonin-permeabilized cells and the following protocol was used throughout the experiments:

1GM (2mM; 0.8mM), 2D (1mM), 3P (2mM), 4S (1M), 5U (titration with FCCP), 6ROT (1mM) and 7Ama (5mM).
RESULTS

CELL VIABILITY
(MTT assay)

Data expressed as a mean ± 95% CI; n=8.

Statistical analysis: one-way ANOVA and Tukey tests
Table 1. Selected respiratory states and respiratory control ratios calculated for breast cancer cells (4T1 cell line)

<table>
<thead>
<tr>
<th>Term</th>
<th>Control</th>
<th>MET</th>
<th>MG</th>
<th>MET+MG</th>
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</thead>
<tbody>
<tr>
<td>OXPHOS I&amp;II</td>
<td>118.53 [90.93-181.38]</td>
<td>98.80 [89.20-133.82]</td>
<td>147.64 [127.40-194.90]</td>
<td>118.81 [90.09-147.47]</td>
</tr>
<tr>
<td>ETS I&amp;II</td>
<td>142.03 [94.67-230.85]</td>
<td>118.01 [101.09-173.11]</td>
<td>174.42 [137.05-255.25]</td>
<td>135.51 [107.42-187.54]</td>
</tr>
<tr>
<td>OXPHOS coupling efficiency</td>
<td>0.85 [0.81-0.87]</td>
<td>0.84 [0.83-0.87]</td>
<td>0.89 [0.86-0.90]</td>
<td>0.83 [0.79-0.86]</td>
</tr>
<tr>
<td>ETS capacity efficiency</td>
<td>0.86 [0.85-0.88]</td>
<td>0.87 [0.86-0.88]</td>
<td>0.89 [0.86-0.90]</td>
<td>0.85 [0.83-0.89]</td>
</tr>
</tbody>
</table>

Data are shown as medians and interquartile ranges, n=6. All values of the oxygen consumption by mitochondria of 4T1 cells, untreated or treated with 1.5 mM metformin (MET), 0.05 mM methylglyoxal (MG) or both (MET+MG), were corrected by ROX (Residual Oxygen). Oxygen consumption was expressed as pmol O₂ per second per 10⁶ cells. There were no statistically significant differences between the tested samples (Friedmann’s test).
Data are shown as medians, interquartile ranges and raw data, n=6; Oxygen consumption was expressed as pmol O₂ per second per 1 million of cells. There were no statistically significant differences between tested samples.
Data are shown as medians, interquartile ranges and raw data, n=6; Oxygen consumption was expressed as pmol O$_2$ per second per 1 million of cells. There were no statistically significant differences between tested samples.
It seems that both metformin and methylglyoxal (used at relatively low concentrations; IC25), when used under in vitro conditions, may not reveal the properties, which could be taken into account when planning anti-cancer therapies aimed at mitochondria as the therapeutic target for these compounds.
Mitochondria-targeted metformins: anti-tumour and redox signalling mechanisms

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2017

24h

metformin

Mito-Met₂

Mito-Met₆

Mito-Met₁₀

Mito-Met₁₂

Mito-Met₂ (n = 2)
Mito-Met₆ (n = 6)
Mito-Met₁₀ (n = 10)
Mito-Met₁₂ (n = 12)
The graph shows the survival fraction (%) on the y-axis and concentration (μM) on the x-axis. Two different conditions are depicted: Met and Mito-Met10.

- For Met, the IC50 is 1.1 μM.
- For Mito-Met10, the IC50 is 1.3 mM.
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