Mitochondrial respiratory complexes as targets of drugs

- Critical importance of protocols: intact vs permeabilised cells -

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ClinicalTrials.gov is a registry and results database of publicly and privately supported clinical studies of human participants conducted around the world.

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643 studies found for: mitochondria
Mitochondrial respiratory complexes as targets of drugs
The example of fibrates and glitazones
Clofibric acid

Gemfibrozil

Bezafibrate
Other intriguing (less known and/or considered) biochemical properties such as:

* Peroxisomes proliferator activity, particularly at hepatocyte level;
* Hepatocarcinogenic effect in rodents;
* Allosteric interaction with human hemoglobin with a consequent reduction of oxygen affinity of the hemoprotein;
* Direct or indirect stimulatory action at the level of genes codifying for cytochrome P450 IV family;
* Myotonic-like activity, which can induce muscular disorders resembling, from the clinical and pathological point of view, the myotonic dystrophy;
* An enhancement of the oxidative burst of leukocytes in vitro;
* An auxinic and herbicide-like activity;
* A debated inhibitory activity of mitochondrial energetics in vitro and in vivo
Moreover, the following points have to be taken in consideration:

* Synthetic PPARs ligands are a very heterogeneous class of amphipatic chemicals which display very similar effects.

* PPAR-α and -γ induce the expression of different gene families related to lipid metabolism (catabolism or storage of fatty acid, respectively).

* Despite their different physiological roles, synthetic ligands for PPAR-α and -γ actually cause similar therapeutic effects in vivo (hypolipidemic and hypoglycemic effects).

* A great number of studies demonstrated that synthetic ligands for PPAR-α and -γ display both a differentiating activity and a carcinogenic role on experimental models.

* Paradoxically, these activities, much alike as their metabolic effects are regarded, do not strictly depend on cell or tissue PPAR isotype expression.
We have cloned a member of the steroid hormone receptor superfamily of ligand-activated transcription factors. The receptor homologue is activated by a diverse class of rodent hepatocarcinogens that causes proliferation of peroxisomes. Identification of a peroxisome proliferator-activated receptor should help elucidate the mechanism of the hypolipidaemic effect of these hepatocarcinogens and aid evaluation of their potential carcinogenic risk to man.

The physiological role and putative natural ligand of PPAR are unknown. The induction of the peroxisomal fatty-acid β-oxidation system by peroxisome proliferators may indicate a role for the receptor in providing a source of energy via fatty acids. Additional possibilities include the regulation of peroxisomal function or cholesterol metabolism, for example by increasing the conversion of cholesterol into bile acids; a step of which is performed mainly, if not exclusively, in the peroxisomes.

*Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, UK.
**Figure 2.** NMR metabolite determinations in supernatant of HL-60 cell cultured for 96 hours in presence of bezafibrate. A dose dependent increase of lactate, acetate and alanin pointed out a compensatory shift to anaerobic metabolism. Results are expressed as the mean ± SEM, calculated from four experiments, each performed in duplicate. The group means were compared by analysis of variance (ANOVA) followed by a multiple comparison of means by Student-Newman-Keuls. $p < 0.05$ was considered significant.

*** = $p < 0.001$, * = $p < 0.05$. 

Figure 6. Cell cultured in presence of bezafibrate consumed more glucose than controls though different cell growth kinetics. HL-60 and Te-671 cultured in presence of bezafibrate, 1 mmol/l and 0.5 mmol/l respectively, showed to significantly increase glucose utilization with respect to basal levels consumed. Results are expressed as the mean ± SEM, calculated from four experiments, each performed in duplicate. The group means were compared by analysis of variance (ANOVA) followed by a multiple comparison of means by Student-Newman-Keuls. p < 0.05 was considered significant. *** = p < 0.001, ** = p < 0.01.
Thiazolidinediones (TZDs or glitazones): Pioglitazone and Rosiglitazone

- **Mechanism of Action**
  - Enhance insulin sensitivity in muscle, adipose tissue
  - Inhibit hepatic gluconeogenesis
  - Perhaps, stabilize beta cell dysfunction

- **Safety and Efficacy**
  - Decrease A1C 1–2%
  - Adverse events: edema, wt. gain, anemia; worry: liver/heart failure

- **Dosing**
  - Initial dose: Pio 15 mg qd, Rosi 2-4 mg qd (rosi can be dosed bid)
  - Maximum effective dose: maximum dose (pio 45 mg qd, rosi 8 qd)
  - Titration frequency: weeks to month(s)

HepG2 cells

Antiproliferative effect

Differentiating effect

Complex I inhibition

Metabolic alterations
No inquiry has been held, no one has apologised, no one’s career has suffered (with the exception of Richard Eastman), and a lot of people are wealthier in consequence. Clinicians and researchers working with diabetes did well out of troglitazone, and some still talk with nostalgic regret of its wasted potential. The most striking thing about the story is that the medical community remained resolutely silent on the subject of patient safety. No prominent physician, anywhere in the world, ever stood up to say that a pill for diabetes is not worth dying for.
Gale EA. Pioglitazone: are rumours of its death exaggerated? 
HepG2 cells

Permeabilized cells

CI resting: basal complex I respiration (glut/Mal +Digit)
CI active: CI respiration activated by excess of ADP (Glut/Mal +Digit + ADP)
CI/CII active: combined CI and CII respiration (glut/Mal +Digit +ADP+ Succ)
HepG2 cells

Intact cells

**Routine**

**Oligomycin**

**FCCP**

**Routine vs respective basal**

**Oligomycin vs respective basal**

**FCCP vs respective basal**
HepG2 cells

ROX intact cells

Respiration (pmoles/sec * 10^6 cells)

Vehicle  Cgz  Gfz  Clo  Bzf

**  *  *  *
HepG2 cells
It could be useful to stress that the outlier data (i.e., ciglitazone) seem to give the statistical significance to the whole correlation, while even eliminating data relative to ciglitazone, a statistically significant linear regression persists between mitochondrial derangement and pK values ($r^2 = 0.9981$, $P<0.03$) and between mitochondrial derangement and log D at pH 8.0 ($r^2 = 0.9998$, $P<0.01$) of the three remaining drugs. In conclusion, the outlier data only reinforce the statistical significance of the correlation that is already present with only the other three drugs, fibrates.
Clinical implications

The observed mitochondrial derangement should be considered with respect to dangerous side effects such as:

- Rhabdomyolysis (fibrates alone or in association with HMGCoA inhibitors - cerivastatin -)
- Heart failure (thiazodilinediones, i.e. troglitazone)
- Acute liver insufficiency (thiazodilinediones, i.e. troglitazone)
- Kidney: water retention
- Myocardial infarction (rosiglitazone)

- CANCER??

Effects related to prolonged treatments?
Ingenuity Pathway Analysis (IPA) to assign differentially expressed proteins to functional networks

<table>
<thead>
<tr>
<th>Tox List</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial Dysfunction</td>
<td>2.74E-04</td>
</tr>
<tr>
<td>Renal necrosis/Cell death</td>
<td>1.51E-03</td>
</tr>
<tr>
<td>Fatty Acid Metabolism</td>
<td>4.62E-03</td>
</tr>
<tr>
<td>Alteration Transmembrane Potential of Mitochondria and Mitochondria membrane</td>
<td>1.27E-02</td>
</tr>
<tr>
<td>Oxidative Stress</td>
<td>1.58E-02</td>
</tr>
</tbody>
</table>

**Mitochondria dysfunction-associated molecules**

ATP5A1, ATP5O, PRDX3, SOD2, VDAC2

**Top Networks**

<table>
<thead>
<tr>
<th>Associated Network Functions</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurological Disease, Skeletal and Muscular Disorders, Hereditary Disorder</td>
<td>51</td>
</tr>
<tr>
<td>Infectious Diseases, Developmental Disorder, Neurological Disease</td>
<td>48</td>
</tr>
<tr>
<td>Cell Morphology, Reproductive System Development and Function, Lipid Metabolism</td>
<td>20</td>
</tr>
<tr>
<td>Cancer, Organismal Injury and Abnormalities, Reproductive System Disease</td>
<td>15</td>
</tr>
<tr>
<td>Psychological Disorders, Antimicrobial Response, Inflammatory Response</td>
<td>5</td>
</tr>
</tbody>
</table>
Cancer stem cells (CSCs), or cancer initiating cells (cancer maintaining cells, stem-like cancer cells, pathogenically relevant cancer cells) are distinct from the bulk of the tumor and they are responsible for long-term maintenance of tumor growth.

This subpopulation acquired some of the characteristics of stem cells to survive and adapt to ever-changing environments (i.e., ability to self-renew and the capacity to produce progenitors that differentiate in more mature cell types).

The precise mechanism of mitochondria damage and its consequence in terms of cellular oxidative metabolism imbalance;

The interrelationships between peroxisome proliferator activity and mitochondrial damage

The compensatory machinery (essentially in terms of HSP induction) implicated in the maintenance of cellular homeostasis and its role in promoting the differentiation state;

The meaning of such a compensatory mechanism in a peripheral modulation of tumour suppressor genes and/or oncogenes expression;

The role of this particular class of differentiating factors in a new definition of dedifferentiation/differentiation processes in cancer.
**HepG2 cells**

**Antiproliferative effect**

**Differentiating effect**

**Metabolic alterations**

Complex I inhibition

<table>
<thead>
<tr>
<th>Hep-G2</th>
<th>Mitochondria damage index</th>
<th>Lactate index</th>
<th>AntiProliferative index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bezafibrate</td>
<td>1.7 ± 0.5</td>
<td>2.8 ± 0.2</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Clofibric acid</td>
<td>2.9 ± 0.9</td>
<td>4.8 ± 0.8</td>
<td>5.3 ± 0.8</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>9.5 ± 2.7</td>
<td>12.5 ± 0.6</td>
<td>8.2 ± 0.4</td>
</tr>
<tr>
<td>Ciglitizone</td>
<td>111.2 ± 17.3*</td>
<td>102 ± 5.2**</td>
<td>102 ± 4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>r^2 = 0.9989</td>
<td>r^2 = 0.9991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p = 0.0001</td>
<td>p = 0.0001</td>
</tr>
</tbody>
</table>

Table II. Quantitative comparison, on molar ratio basis (drug activity/[drug] x 100), between different PPARs ligands and gemfibrozil in terms of inhibition of NADH cytochrome c reductase activity, lactate and antiproliferative index on Hep-G2 cell line. Results are expressed as the mean ± SEM, calculated from four experiments, each performed in duplicate. The group means were compared by analysis of variance (ANOVA) followed by a multiple comparison of means by Student-Newman-Keuls. p < 0.05 was considered significant. * = p < 0.05; ** = p < 0.01.
<table>
<thead>
<tr>
<th>Ciglitizone [μM]</th>
<th>Mitochondria damage index</th>
<th>Lactate</th>
<th>AntiProliferative index</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>16.4 ± 0.7</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>32.0 ± 0.1*</td>
<td>41.3 ± 0.8 **</td>
<td>46.0 ± 25.9**</td>
</tr>
<tr>
<td>33</td>
<td>56.0 ± 1.1**</td>
<td>44.7 ± 0.6**</td>
<td>52.0 ± 24.9**</td>
</tr>
<tr>
<td>50</td>
<td>61.0 ± 0.2**</td>
<td>50.9 ± 5.2**</td>
<td>52.0 ± 8.0**</td>
</tr>
</tbody>
</table>

|                 | r² = 0.9266               | p = 0.037 | r² = 0.7938             | p = 0.1091 |

Table III. Dose dependent effect of ciglitizone in terms of inhibition of NADH cytochrome c reductase activity, lactate production and antiproliferative index on Hep-G2 cell line. Results are expressed as the mean ± SEM, calculated from four experiments, each performed in duplicate. The group means were compared by analysis of variance (ANOVA) followed by a multiple comparison of means by Student-Newman-Keuls. p < 0.05 was considered significant. * = p< 0.05; ** = p < 0.01.

Pioglitazone purges quiescent CML stem cells


Pioglitazone induces complete and sustained molecular response (CMR) in CML patients
But this is a different mitochondrial story....

Thank you
Thiazolidinediones

- Possible side effects:
  - Edema (particularly peripheral edema)
  - Weight gain
  - Headache, Weakness
  - Nausea, vomiting, abdominal pain
  - Bone fracture
  - Increase the risk of heart failure
  - Liver problems (RARE)
  - Pioglitazone associated with small risk of bladder cancer
  - Potential myocardial infarctions (rosiglitazone)