Old pharmaceuticals with new applications: the case studies of lucanthone and mitoxantrone

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Graphical Abstract

Design of P-glycoprotein inhibitors with antitumor activity

Are mitoxantrone metabolites responsible for their cardiotoxicity?

Mitoxantrone (MTX)

Naphthoquinoxaline

Naphthoquinoxaline metabolite of mitoxantrone is less cardiotoxic than the parent compound and it can be a more cardiosafe drug

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sponsors: MDPI pharmaceuticals
Abstract: The recent overview of pharmaceutical companies' R & D programs has been undergoing some changes, especially due to increased immunopharmacology-based treatments. A trend that has also been growing is the search for new activities that may be demonstrated by drugs already used in therapeutics.
We will give examples of antitumor small molecules lead compounds obtained in our research group that arise from two existing drugs, lucanthone and mitoxantrone (MTX). Lucanthone was the antitumor model used to design inhibitors of P-glycoprotein with antitumor activity. Very recently we engaged a project that intend to understand the influence of metabolites in the cardiotoxicity of an antitumor drug, MTX. Studies on cardiotoxicity of a synthetized metabolite, naphthoquinoxaline (NAPHT) revealed that the parent drug, MTX, caused a higher disruption in the energetic pathways in a cardiac model in vitro. Therefore, this metabolite should be regarded as a good option for a safer anticancer therapy since it is less cardiotoxic than MTX.
The case studies presented herein are expected to contribute to a recent trend in drug discovery, with the involvement of old pharmaceuticals.

Keywords: old drugs; lucanthone; mitoxantrone; P-glycoprotein; metabolism
Special Issue "Old Pharmaceuticals with New Applications"

In recent years, we have experienced a surge of interest in drug repositioning. There is a trend in finding new uses for existing drugs, especially in diseases where there is an unmet clinical need such as neglected and orphan diseases. Another opportunity is developing novel applications using a combination of old drugs.

“The most fruitful basis for the discovery of a new drug is to start with an old drug” goes a famous statement from Sir James Black, which has received many adherents this century, not only in finding new applications but also looking for the unexploited potential of old drugs as starting points for molecular modifications.

The journal Pharmaceuticals invites both reviews and original articles shedding light on the challenges and opportunities of using old pharmaceuticals in drug discovery. Topics include: drug repositioning, selective optimization of side effects, drug metabolites as sources of new drugs, old drug combinations, beyond pharmaceuticals applications.
Case-study: Lucanthone

Lucanthone

Antischistosomal introduced in therapy, in 1945

Withdraw due to mutagenic side effects

Moiety associated to cardiotoxic effects

Phase I dose-escalation study of lucanthone in patients with recurrent malignant gliomas receiving temozolomide

APE-1/ BER

APE-1 - apurinic-apyrimidinic endonuclease
BER – base excision repair

DNA repair systems

MGMT/Direct repair

MGMT - Methyl guanine methyl transferase

Design of P-glycoprotein inhibitors with antitumor activity

Pharmacophoric moiety for antitumor activity

Pharmacophoric moiety for P-glycoprotein modulation

~ 1000 designed thioxanthones (Tx)

Docking

Molecules with the best scores

(III) Synthesis of thioxanthones (Tx)

Hundreds...
Cell growth inhibition (Sulphorhodamine-B assay)

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<th>Compound</th>
<th>$GI_{50}$ (K562) (µM)</th>
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<tr>
<td>TxA1</td>
<td>1.90 ± 0.15</td>
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<tr>
<td>Tx141</td>
<td>3.00 ± 0.48</td>
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<tr>
<td>Tx34</td>
<td>3.72 ± 1.47</td>
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<tr>
<td>TxOH131</td>
<td>4.38 ± 0.44</td>
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<tr>
<td>TxOMe</td>
<td>4.47 ± 1.93</td>
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<tr>
<td>Tx18</td>
<td>4.81 ± 4.21</td>
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<tr>
<td>Tx127</td>
<td>12.98 ± 0.36</td>
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<tr>
<td>TxAc</td>
<td>13.57 ± 2.96</td>
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<td>Tx131</td>
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<td>Tx41</td>
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<tr>
<td>Tx104</td>
<td>16.50 ± 3.06</td>
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<td>Tx129</td>
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<tr>
<td>TxA3</td>
<td>H</td>
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<tr>
<td>Tx54</td>
<td>H</td>
</tr>
<tr>
<td>Tx105</td>
<td>H</td>
</tr>
<tr>
<td>Verapamil</td>
<td>H</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>0.06 ± 1.27</td>
</tr>
</tbody>
</table>

$GI_{50}$ values for new thioxanthonic compounds in K562 (sensitive) cell line

Six new compounds $GI_{50} < 10\mu$M

No significant effect on MRC-5 cell line (non-tumor cells, trypan blue)

TxOMe induced an S-phase cell-cycle arrest; the six Tx induced a decrease of the G2/M phase

Most Tx derivatives increased cellular apoptosis
Rh-123 accumulation assay

- **Verapamil**, **Quinidine** and **Mibebradil** (known Pgp inhibitors): increase the accumulation of Pgp substrate Rh123

- **TXA3, TXA4, TX54, TXOH, TXAc, TX87**: effect compatible with Pgp activation

- **TX48, TX53, TX86**: effect compatible with Pgp inhibition ~ Quinidin
Case-study: Lucanthone

Antitumor agent

Molecules 2016, 21, 1343

Biochemical Pharmacology 2012, 83 (1), 57–68.

Lucanthone

P-glycoprotein inhibitor

P-glycoprotein activator

Archives of Toxicology, 2015, 89(10):1783-800.
Case-study: Mitoxantrone (MTX)

approved in 1987 as antitumor drug and in 2002 for use in multiple sclerosis

MTX-induced cardiotoxicity

- **Adverse effects**: early and late cardiotoxicity.
- **Cardiotoxicity** affects up to 18% of MTX-treated patients, being multiple sclerosis patients more susceptible.
- **Maximum recommended cumulative doses**:
  - Cancer patients: 140 mg/m²
  - Multiple sclerosis patients: 100 mg/m²

Mechanisms involved in cardiotoxicity: largely unknown.

Drug repurposing
Synthesis of drug metabolites to study their toxicity


Carboxilic acid metabolites of MTX

Anticancer activity: inactives
Cardiotoxicity: n.a.

<table>
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<tr>
<th>Compounds</th>
<th>R₁</th>
<th>R₂</th>
</tr>
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<tbody>
<tr>
<td>Monocarboxylic acid of MTX</td>
<td>COOH-</td>
<td>CH₂OH-</td>
</tr>
<tr>
<td>Dicarboxylic acid of MTX</td>
<td>COOH-</td>
<td>COOH-</td>
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</table>

Mitoxantrone (MTX)

Conjugation

Naphthoquinoloxaline of MTX

Anticancer activity: active
Cardiotoxicity: n.a.

Metabolization S9 and microsomal products of MTX

Anticancer activity: active
Cytotoxicity (namely cardiotoxicity): higher

MTX conjugates

Anticancer activity: n.a.
Cardiotoxicity: n.a.
Synthesis of drug metabolites to study their toxicity

Synthesis and purification of MTX-naphthoquinoxaline metabolite

MTX (1) + HRP, H₂O₂ + ammonium acetate buffer (pH 5) → NAPHT (2)

HRP – Horseradish peroxidase

HPLC chromatogram of NAPHT (2) crude

UV-Vis spectra of MTX (1)

UV-Vis spectra of NAPHT (2)

HPLC chromatogram of NAPHT (2)

Analytical

95% of purity

- New chromophore was formed
- HRMS m/z 443.19313

Representative HPLC chromatograms (λ= 254 nm, C18, isocratic system 3:7 of eluent B within 30 min).
Studies on naphthoquinoxaline (NAPHT) cardiotoxicity

- MTX causes higher cellular damage in H9c2 differentiated cells than does NAPHT.
- MTX and NAPHT produce mitochondrial dysfunction in differentiated H9c2 cells, although less pronounced for NAPHT.
- MTX causes a greater loss of cellular membrane integrity.
- MTX caused a more severe lysosome uptake dysfunction.
- MTX increased intracellular ATP levels and lactate levels, whereas its metabolite did not change those parameters.

3-Methyladenine, an autophagy inhibitor, partially protected against lysosomal uptake dysfunction.

The parent drug, MTX, caused a higher disruption in the energetic pathways in a cardiac model in vitro.

Previous data has shown that NAPHT can have a potential role on MTX anticancer effects.

NAPHT can be a more cardiosafe drug in anticancer therapy.

Synthesis of drug metabolites to study their toxicity

Synthesis of carboxylic acid derivatives of MTX

Synthesis of acetoxy derivatives of MTX
Opportunities with existing drugs

One of several examples...

- Sorafenib (7) is known to induce acute coronary symptoms including myocardial infarction in 2.9% of patients.
- No assessment of the potential cardiotoxicity of metabolites was done so far, to the best of our knowledge.

Conclusions

**Structure-based design**
- Lucanthone
- Metabolism-based design
- Mitoxantrone

- **Highly active models**
- Surpassed clinical trials
- Examples of drug repurposing

- Most active leads
- Mee better drugs?

*To be continued*

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Acknowledgments

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