A combined biological and mathematical approach for studying the circadian control of the anticancer drug Irinotecan pharmacokinetics-pharmacodynamics

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Introduction: Chronotherapy

• Chronoefficacy/chronotoxicity of many anticancer drugs have been shown in experiments on mice.
• Chronotherapeutic schemes of infusion of the drug have been designed for mice, and then adapted for humans.

Administration Scheme currently used by Francis Lévi’s INSERM team U 776:

- **Folinic Acid**
  - 300 mg/m²/d

- **OxaliPt**
  - 25 mg/m²/d

- **5-FU**
  - 600 - 1100 mg/m²/d

Infusion over 5 days every 3 weeks
Introduction: Circadian Rhythms

Results of chronotherapeutics versus constant administration

Metastatic colorectal cancer
(Treated with Folinic Acid, 5-FU, Oxaliplatin)

<table>
<thead>
<tr>
<th>Infusion flow</th>
<th>CONSTANT</th>
<th>CHRONO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral mucositis gr 3-4</td>
<td>74%</td>
<td>14%</td>
</tr>
<tr>
<td>Neuropathy gr 2-3</td>
<td>31%</td>
<td>16%</td>
</tr>
<tr>
<td>Responding rate:</td>
<td>30%</td>
<td>51%</td>
</tr>
</tbody>
</table>

Chronotherapy improves the responding rate to treatment and decreases the toxicity compared to constant infusion of the drugs.
Introduction: Circadian Rhythms

Question:
Can such drug delivery schedules be improved?
Focus on the anticancer drug Irinotecan

Aims:
- explain at a molecular level CPT11 chronotoxicity/chronoefficacy.
- find optimal scheme of administration of CPT11, for a given circadian profile.

Means:
1. Cell culture
2. Mathematical Modeling
1. Irinotecan Pharmacokinetics/Pharmacodynamics
Irinotecan (CPT11) is a pro-drug, i.e. it has to be activated into SN-38 which is 1000-fold more efficient. This reaction is catalysed by Carboxylesterases (CES).

SN-38 is then glucuronided into SN-38G which is inactive. This reaction is catalysed by the enzyme UGT1A1.
CPT-11, SN-38 and SN-38G are transported outside of the cell by ATP-Binding Cassette (ABC) transporters, which are active efflux pumps.
Irinotecan is an inhibitor of Topoisomerase 1. What is TOP1?

TOP1 is a nuclear enzyme which is present in healthy cells and aims at relaxing the supercoiled DNA:

1) TOP1 Wraps the supercoiled DNA:
2) TOP1 cuts one DNA strand so that it can rotate
3) TOP1 reconnects the broken strand.

---

![Diagram of TOP1 enzyme action on DNA](image)

- **TOP1** wraps the supercoiled DNA.
- TOP1 cuts one DNA strand to allow rotation.
- TOP1 reconnects the broken strand to relax the DNA.
Irinotecan is an **inhibitor of TOP1**: 

- Irinotecan prevents TOP1 from reconnecting the DNA broken strand, creating reversible TOP1/DNA/Irinotecan complexes.

- The collision between those complexes and replication forks or transcription mechanisms creates DNA double-stranded breaks, which can be lethal for the cell.
1. Irinotecan Pharmacokinetics/Pharmacodynamics
2. Studying Irinotecan in cell culture
3. A Mathematical Model including Circadian Rhythms

Summary

CPT11\text{out} \quad \text{ABC transporters} \quad \text{CPT11}_{\text{in}} \quad CES \quad SN38_{\text{in}} \quad UGT1A1 \quad SN38G

DNA \quad TOP1 \quad \text{DNA/TOP1 Complex} \quad \text{Reversible Complex} \quad \text{Double-Stranded Break} \quad \text{Cell Cycle Arrest / Apoptosis}

Replication, Transcription
1. Studying Irinotecan in cell culture
Experiments on Caco-2 cells (human epithelial colorectal adenocarcinoma cells) have been performed.

A Petri Dish

The cells stick to the bottom of the dishes.

The extracellular medium is added on top of the cells

Caco-2 cells under microscope
Exposure of Caco2 cells to CPT11 (140μM) during 48H

Measurement of [CPT11] and [SN38] by HPLC

<table>
<thead>
<tr>
<th>Extracellular concentration</th>
<th>Intracellular concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CPT11</strong></td>
<td></td>
</tr>
<tr>
<td><img src="image1" alt="CPT11 extracellular graph" /></td>
<td><img src="image2" alt="CPT11 intracellular graph" /></td>
</tr>
<tr>
<td><strong>SN38</strong></td>
<td></td>
</tr>
<tr>
<td><img src="image3" alt="SN38 extracellular graph" /></td>
<td><img src="image4" alt="SN38 intracellular graph" /></td>
</tr>
</tbody>
</table>
Seric shocks (ie. exposing cells to a large amount of nutrients during 2 hours) synchronize the circadian clock of the cells which oscillate in synchrony.

Circadian clock oscillate in Caco-2 cells:

mRNA Curve Fitting:

\[
[mRNA](t) = R + S e^{\lambda t}(1 + \epsilon \cos\left(\frac{2\pi}{T} + \phi\right))
\]
# Experimental results on Caco-2 cells

1. Irinotecan Pharmacokinetics/Pharmacodynamics
2. Studying Irinotecan in cell culture
3. A Mathematical Model including Circadian Rhythms

<table>
<thead>
<tr>
<th>Topoisomerase 1 (Drug Target)</th>
<th>CES2 (Activation Enzyme)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Graph" /></td>
<td><img src="image2.png" alt="Graph" /></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>UGT1A1 (Deactivation Enzyme)</th>
<th>ABCB1 Transporter</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3.png" alt="Graph" /></td>
<td><img src="image4.png" alt="Graph" /></td>
</tr>
</tbody>
</table>
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Experimental results on Caco-2 cells

Irinotecan Chronoefficacy

Difference in Apoptosis three days after one-hour exposition
3. A Mathematical Model including Circadian Rhythms
Mathematical Modeling

CPT11\textsubscript{out} \rightarrow \text{ABC transporters} \rightarrow \text{CPT11}\textsubscript{in} \rightarrow \text{CES} \rightarrow \text{SN38}\textsubscript{in} \rightarrow \text{UGT1A1} \rightarrow \text{SN38G} \rightarrow \text{DNA/TOP1 Complex} \rightarrow \text{Reversible Complex} \rightarrow \text{Double-Stranded Break} \rightarrow \text{Cell Cycle Arrest / Apoptosis}
Mathematical Modeling

➢ One differential equation for each variable.

➢ Equation for \([\text{CPT11}_{\text{out}}]\):

\[
\frac{d[\text{CPT11}_{\text{out}}]}{dt} \frac{V_{\text{out}}}{V_{\text{in}}} = -k_{\text{uptakeCPT}} \frac{V_{\text{out}}}{V_{\text{in}}} [\text{CPT11}_{\text{out}}] + \frac{V_{\text{effCPT}}[ABC][\text{CPT11}_{\text{in}}]}{K_{\text{effCPT}} + [\text{CPT11}_{\text{in}}]}
\]

\(\uparrow\)
Change over time

\(\uparrow\)
CPT11 cell uptake (passive)

\(\uparrow\)
CPT11 cell efflux (active ABC transporters)

\([\text{CPT11}_{\text{out}}]\) = CPT11 extracellular concentration

\([\text{CPT11}_{\text{in}}]\) = CPT11 intracellular concentration

\(V_{\text{out}}\) = volume of extracellular medium

\(V_{\text{in}}\) = volume of intracellular medium

\(k_{\text{uptakeCPT}}\) = speed of CPT uptake

\(V_{\text{effCPT}}, K_{\text{eff}}\) = Michaelis Menten parameters for CPT efflux
Mathematical Modeling

Complete PK-PD model:

\[
\begin{align*}
\frac{d[C\text{PT11}_{\text{out}}]}{dt} &= -k_{\text{uptakeCPT}} \frac{V_{\text{out}}}{V_{\text{in}}} [C\text{PT11}_{\text{out}}] + \frac{V_{\text{effCPT}}[ABC][C\text{PT11}_{\text{in}}]}{K_{\text{effCPT}} + [C\text{PT11}_{\text{in}}]} \\
\frac{d[C\text{PT11}_{\text{in}}]}{dt} &= k_{\text{uptakeCPT}} \frac{V_{\text{out}}}{V_{\text{in}}} [C\text{PT11}_{\text{out}}] - \frac{V_{\text{effCPT}}[ABC][C\text{PT11}_{\text{in}}]}{K_{\text{effCPT}} + [C\text{PT11}_{\text{in}}]} - \frac{V_{\text{CPT-SN}}[C\text{PT11}_{\text{in}}]}{K_{\text{CPT-SN}} + [C\text{PT11}_{\text{in}}]}
\end{align*}
\]

\[
\begin{align*}
\frac{d[SN38_{\text{out}}]}{dt} &= -k_{\text{uptakeSN}} \frac{V_{\text{out}}}{V_{\text{in}}} [SN38_{\text{out}}] + \frac{V_{\text{effSN}}[ABC][SN38_{\text{in}}]}{K_{\text{effSN}} + [SN38_{\text{in}}]}
\end{align*}
\]

\[
\begin{align*}
\frac{d[SN38_{\text{in}}]}{dt} &= k_{\text{uptakeSN}} \frac{V_{\text{out}}}{V_{\text{in}}} [SN38_{\text{out}}] - \frac{V_{\text{effSN}}[ABC][SN38_{\text{in}}]}{K_{\text{effSN}} + [SN38_{\text{in}}]} + \frac{V_{\text{CPT-SN}}[C\text{PT11}_{\text{in}}]}{K_{\text{CPT-SN}} + [C\text{PT11}_{\text{in}}]}
\end{align*}
\]

\[
\begin{align*}
\frac{d[SN38G]}{dt} &= \frac{V_{\text{SN-SNG}}[UC\text{T}][SN38_{\text{in}}]}{K_{\text{SN-SNG}} + [SN38_{\text{in}}]} - k_{\text{fC}}[\text{TOP1}] [SN38_{\text{in}}] (\text{DNA}_{\text{tot}} - [\text{COMPL}]) + k_{\text{rC}}[\text{COMPL}]
\end{align*}
\]

\[
\begin{align*}
\frac{d[\text{COMPL}]}{dt} &= k_{\text{fC}}[\text{TOP1}] [SN38_{\text{in}}] (\text{DNA}_{\text{tot}} - [\text{COMPL}]) - k_{\text{rC}}[\text{COMPL}]
\end{align*}
\]
Mathematical Modeling

Simulation: choosing the right circadian time to expose cells
Conclusion and future work

- More data are expected (CPT-11, SN-38, SN-38G transport kinetics, reversible complexes formation/dissociation, protein level…)

- Once the mathematical model is calibrated and validated (by other cell culture experiments), it will be used to define a **theoretically optimal scheme** for exposition of Caco-2 cells to Irinotecan.

- Future: this study at the cell population level may then be integrated into a whole-body approach (modeling tissular CPT11 PK-PD) for the mouse.
Aim: design theoretically optimal scheme of administration for the three mouse chronotoxicity classes (cf. C. Ahowesso work).

Mean: Whole Body PK PD Model based on the mathematical model of cell culture (cf. H. Gayrard work)