Applications of in silico modelling in drug metabolism study in pharmaceutical research

Examples on the combined application of experimental data and in silico modelling

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Lecture University of Basel 20-11-2020
Introduction and aim of the talk

- The aim of the talk is that to report the basic activities necessary for a full understanding of the human drug metabolism
- I will provide some basics concepts of metabolism and the respective modelling which can be applied for the different kind of studies
- In addition, I will try to contextualize the talk using the experience which I have gained during my educational period
Metabolism Key-Concepts

- Metabolites may be toxic for different organs in the body
- Interaction with other xenobiotics (DDI)
- Inactive towards biological target
- More active than substrate towards biological target => **PRODRUG**

**DRUG CANDIDATE** → **METABOLISM**

- Decreased drug bioavailability
- Decreased therapeutic effect

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Drug metabolism study in a pharmaceutical company context

- Identify Target
- Identify Compounds
- Establish Activity
- Select Clinical Candidates
- Test Safety and PK
- Human Clinical Trial Phase I
- Human Clinical Trial Phase II
- Human Clinical Trial Phase III
- Human Clinical Trial Phase IV

Discovery Stage

Development Stage

- Late discovery stage
- Early drug development
- Pre-clinical development

Modelling & exp. data

Semi-quantitative metabolism determination
Quantitative approach with a limited in vivo transferability
Quantitative approach for pre-clinical evaluation

Topic of my activity

Aldehyde Oxidase
UGT2B10
QoC

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Aldehyde Oxidase (AOX)

- Present in several organisms (animals, vegetables, fungus and bacteria…)
- Mainly present in animal cytosol liver
- A Molybdo-flavoprotein
- Homodimeric enzyme with 3 domains
- Uses $O_2$ as final electron acceptor
- There is a wide intra and interspecific variability

C. Coelho et al. *Nature Chemical Biology* 11, 779–783, **2015**

Garattini & Terao *Expert Opin. Drug Discov.* 8, 641-654, **2013**
Pryde et al. *J. Med. Chem.* 53, 8441–8460, **2010**
AOX- promoted reactions

Aldehyde oxidation

\[ R^\cdot H \xrightarrow{\text{AOX}[1]} R^\cdot COH \]  

\[ \text{Vanillic acid} \xrightarrow{\text{AOX}} \text{Vanillic acid} \]

Aza-aromatic oxidation

\[ \text{R = EDG, EWG} \]

\[ \text{Carbazepine} \xrightarrow{\text{AOX}[1]} \text{Carbazepine} \]

Amide hydrolysis

\[ R^\cdot \text{N}_2R_1 \xrightarrow{\text{AOX}[2]} R^\cdot \text{OH} + \text{HN}_2R_1 \]

\[ \text{GDC-0834} \xrightarrow{\text{AOX}} \]  

\[ \text{GDC-0834 M1} + \text{GDC-0834 M3} \]

My work was focused on these reactions because:
- Several drugs contained aza-aromatic scaffolds
- There are about 35% of molecules with amide functionality in DrugBank

Many activities for one purpose

Aim: Purely in silico prediction of AOX substrate. Is the compound AOX substrate?

- Compound Synthesis
- External DBs
- In vitro assays with HLC
- LC-MS/MS analysis
- Chromatogram analysis
- Results analysis
- Software development
- Model validation

Peak Area

And metabolite detection (+16 m/z) for the oxidation

Semi-quantitative

Drug/Enzyme affinity + Reactivity

How we can describe the molecular structure of a drug in silico?
Molecular description

- **Molecular structure**
  (SMILES, SDF…)

**Application**: Filter out compounds having a desired functional group

Database with 10 000 molecules

**Query in SMARTS**

[#6][CX3](=O)[#6] → All ketones

- **Global description**

Database with 10 000 molecules

**ADME predictor software** → LogP Ranking

**Application**: Estimation of logP, logD
From the total hydrophobic/hydrophilic domain

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Molecular description

Application of the global molecular description in drug Metabolism

Does the global description of the molecule work to study target-molecule affinity?

VolSurf

VolSurf

logP cannot discriminate substrate and non-substrate of h-AOX

Molecular Similarity (Example)

Using the pure molecular description they seem to be quite different (e.g. no common functional group)

They have several common interactions

In medicinal chemistry these models are mostly base on Molecular mechanics

Application of classical mechanics to molecular systems
Molecular description

Application of the global molecular description in drug Metabolism

Does the global description of the molecule work to study target-molecule affinity?

VolSurf

logP cannot discriminate substrate and non-substrate of h-AOX

Local interactions with the target make a huge difference

Molecular Similarity (Example)

Repaglanide

In medicinal chemistry these models are mostly base on Molecular mechanics

Application of classical mechanics to molecular systems

Molecule X

Molecule Y

Using the pure molecular description they seem to be quite different (e.g. no common functional group)

Solid MIFs -> Repaglanide

Wireframe MIFs -> Molecule Y

They do not have common interactions

Molecular Interaction Fields: Applications in Drug Discovery and ADME Prediction, Volume 27
Molecular description

**Grid Method**

- Hydrophobic
- Hydrogen bond donor
- Hydrogen bond acceptor
- Shape

\[ E = E_{covalent} + E_{noncovalent} \]

The molecule into the box is flexible (conformations are considered)

All points in the grid are used for mapping the molecule

Same approach can be performed for large molecules as protein
Focus on enzymatic reaction

It can be approximated using ab initio methods in non-redundant chemical fragments.

\[ R_i \] is the reactivity of the atom \( i \)-th in the appropriate reaction mechanism. It can be approximated using ab initio methods in non-redundant chemical fragments.
Data mining

Analysis results: SoM exposition

Experimental substrate

Reactive Site of Metabolism (SoM)

Experimental non substrate

Correct exposition

Same principle of docking but the information are collapsed in 4 high informative points (pharmacoporic points)

Wrong exposition

MoCo

Proc Natl Acad Sci U S A 2017 Apr 18;114(16):E3178-E3187
This function is considered to be an approximation of the free energy of the process including substrate-enzyme interaction where exposure (E) and reactivity (R) are opportunely weighted (we and wr, respectively).

*J. Med. Chem., 61, 360–371, 2018*
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UGTs and glucuronidation reaction

**Mechanism reaction**

\[
H-N:R + S_{N2} \rightarrow \text{UDPGA}
\]

- **R** = H, Aliphatic, Ar

**Human UGT isoforms**

- **# isoforms**: 19 isoforms
- **Subfamilies**:
  - 1A
  - 2A
  - 2B

**h-UGT2B10**

- 23 known UGT2B10 substrates from the DDI DB of the Univ. of Washington
- Roche clinical candidate: RO5263397
- 3D structure of UGT2B10 is not available
- No valid experimental protocol to assess UGT2B10 selective substrate

**N-gluc.**

- **19 isoforms**
  - UGT1A4 & UGT2B10
  - 1) Liver
  - 2) Gut
  - 3) Kidney

**Where**

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**UGT2B10: bullet points**

**Aim:** find new selective UGT2B10 substrates

**Why is this project useful in the pharmaceutical field?**

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A UGT2B10 Splicing Polymorphism Common in African Populations May Greatly Increase Drug Exposure


Roche Pharma Research and Early Development, Roche Innovation Center Basel, Basel, Switzerland (S.F., H.K., P.S., D.T., R.D.N., M.C.H., O.S., V.A.I.); and Pharmaceutical Chemistry, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland (M.F., N.M.)

Received September 26, 2014; accepted December 10, 2014

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Aim: find new selective UGT2B10 substrates

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**Clinical candidate against Schizophrenia disease**

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Volunteer with poor UGT2B10 expression

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Normal metabolizer

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Journal of Pharmacology and Experimental Therapeutics 2015, 352 (2) 358-367
Application of in vitro and in silico modelling

There are two broad categories of computational techniques for virtual screening: Structural Based and Ligand Based

**Ligand-based.** It is based on that similar chemical-physical features implicate a similar target affinity. IUPAC defines a pharmacophore to be "an ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response"

Glossary of terms used in medicinal chemistry (IUPAC Recommendations 1998)

**Ligand based - Method used for UGT2B10 project**

**Known UGT2B10 substrates used as templates**

Repeated for all 140 screened compounds and for all 6 templates

**Steps**

578 Known UGTs substrate from DDI Washington database

Remove all substrates without Reactive N

Templates MIFs

140 screened molecules MIFs for max. 25 conf.

Template – screened compound MIFs superimposition

Score per each MIFs probe

Ranking using the HDRYN1 probe (combination of 3 probes)

19 commercial available drugs were selected and purchased

Pre-filtering (SMARTS)
Final results

A frequency number of 1 is added for each screened compound in the 20 top rank of each LB with the 6 templates.

Example

- Clozapine
- Template: Amitriptyline, Cyclizine, Desloratadine, RO5263397, Nicotine, Dexmedetomidine

Frequ. Number = 4

In the top 20
NO in the top 20
Example: Results from LB-VS

578 Known UGTs substrate from DDI Washington database

Remove all substrates without Reactive N

Template MIFs

140 screened molecules MIFs for max. 25 conf.

Template – screened compound MIFs superimposition

Score per each MIFs probe

Ranking using the HDRYN1 probe (combination of 3 probes)

19 commercial available drugs were selected and purchased

Good similarity

Candidate: Cyproheptadine

For clarity the MIFs of the ligands are switched off

Best 10 poses

Low similarity

Candidate: Dihydrocodeine

Top 20 in RO5263397, Desloratadine, and Cyclizine

Never in the top 20
Outcome of the project: new drugs potentially subjected to UGT2B10 polymorphism

\( f_{m}(\text{UGT2B10}) \) and potential polymorphism exposure

Important findings in pharma research!!!
Quantitative Modelling in DMPK

Bachelor Thesis

University of Perugia
Drug metabolism and Molecular Modelling

Master Thesis

University of Perugia
Synthesis in Organic Chemistry
Drug Metabolism and Molecular Modelling

Young Researcher

Molecular discovery Ltd
Drug Metabolism and Development of software for the identification of side products from chemical processes (LC-MS/MS data and NMR)

PhD

University of Perugia and Roche
Drug Metabolism and Molecular Modelling – Highthroughput systems (Roche)

Postdoc

Roche and University of Manchester
DMPK modelling for in vitro data processing and data optimization in vitro assay with Liver/Gut-Liver OoC

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Topic of my activity

UGT2B10
Application of modelling for complex OoC

**Aim:** Application of high physiological system as OoC (Organ-on-a Chip) for DMPK investigation

- Seeded hepatocytes
- Microflow to mimic the physiological blood flow
- High cell longevity

**Single tissue (liver OoC)**
- Long incubation (compound with low metabolic turnover)
- DDI study
- fm estimation

**Multi-tissue (Gut-Liver OoC)**
- First pass metabolism (GI)
- Intestinal absorption
- Hepatic metabolism
- Intestinal and liver cross-talking (high physiological environment)

Better IVIVE
Complexity needs a mathematical deconvolution

Gut and Liver OoC

Compartmental model is described by ODEs

Simple PBPK

Example: the importance of the model description

$P_{\text{app.gut}} = 30$-fold

Tsamandouras-Chen conditions are met

General model

Tsamandouras-Chen model

Same profiles in all compartments regardless of the model

Tsamandouras-Chen conditions are NOT met

General model

Tsamandouras-Chen model

Only the general model represents the real profile
Acknowledgment

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