Drug Metabolism and Pharmakokinetics 101

Transcribed by MMF

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1 Preface

A DMPK department is at its core a **service**. A drug candidate is synthesised by chemists; afterwards it is DMPK's task to predict e.g. the first dose for testing, the safe dose range, or whether a chemically altered or a different candidate might be better.

Results matter a lot in DMPK, because you are working for a **company**, after all. However, there is also **an academia-like aspect** to it, because studies are conducted, and results need to and will be published.

2 Drug Metabolism

The metabolism matters a lot in DMPK. When a drug is taken, the metabolism alters it; and the interaction between drug and metabolism determines the drug's usefulness, e.g. by affecting its half-life time. This is determined by how often the drug needs to pass through the liver again to get completely metabolised.

When a drug is taken for the first time, it undergoes **first-pass metabolism**[1]**.** This determines how much of the compound will be absorbed into the blood via the intestines, portal vein, liver and hepatocytes. One can distinguish two different phases of the first-pass metabolism.

During **first phase**, the drug is made more water-soluble for absorption into the blood and excretion. This is done e.g. by adding an -OH group, or by demethylisation. Both require NADPH as cofactor like many other first phase reactions do. Some very rare reactions like aldehydeoxidase, however, do not. Multiple different first-phase reactions can act upon a drug. Enzymes of the first phase might be missing in some patients, however they are more often affected by **polymorphisms** instead. These can have very large effects, e.g. see polymorphisms of CYP 2D6.

During **second phase**, other modifications are applied to increase solubility even more. E.g. a sugar is added (cofactor UDPGA – UDP plus glucoronic acid) or sulfate is added (cofactor PAPS). The effect of this is huge compared to the effect of first phase transformations: Consider Lipinski's rule of five[2], stating that molecules above 500g/mol generally cannot get into cells properly; adding a large group like a sugar changes this and makes it more soluble for excretion. Second phase does not happen to all drugs; additionally, some people may **lack** certain enzymes responsible for second phase reactions.

3 Studying the Drug Metabolism

The enzymes which act on a drug are mostly contained in the ERs of the liver. These are primarily **Cytochromes (CYPs)**, among which the **CYP450 superfamily** is especially important. CYPs can also be elsewhere, e.g. 2A2 in lung metabolising nicotine or 2C9 in the brain metabolising modafinil, however the bulk of them is in the liver.

[1] If a drug is taken again for maintenance, its metabolic transformations might differ, e.g. due to enzyme induction.

[2] https://en.wikipedia.org/wiki/Lipinski%27s_rule_of_five

The **role of individual CYPs** is not always straightforward.

1. **CYP3A4** is of utmost importance as it interacts with basically everything (and can even act twice in parallel due to its large active center). Usually, it is studied by using a steroid such as testosterone, as well as midazolame as positive control with a good intermediate half-life.

2. **CYP2D6** seems to have a tendency to act upon substances with an N-Methyl group, but lots of exceptions apply.

3. In contrast, **CYP2E1** is nicely predictable as it only attaches to small planar molecules such as paracetamol. Because of this specificity, its practical importance can be low.

4. **Other important CYPs** include 2C9, 2C19, C8, 1A2, 2C8, 2D6, 1A2.

To study drug metabolism in the lab, we use **liver microsomes**. These are *heterogenous vesicle-like artifacts (approx. 20-200 nm diameter) re-formed from pieces of the endoplasmic reticulum (ER) when eukaryotic cells are broken-up*[3]. They are *concentrated and separated from other cellular debris by differential centrifugation*[4].

A practical note: Some drugs are very lipophile, so they tend to come in DMSO. **DMSO, however, interferes with some CYPs, especially 2E1**. Hopefully, you can dilute it enough to use only water, however this is very unlikely. Thus, you titrate as low as possible starting from acetonitrile or methanol by adding water.

To **study the degradation of a drug by CYPs** over time, we mix the microsomes with the drug, start the reaction by adding NADPH, and terminate it after some time with methanol, which precipitates proteins. In addition to the methanol, we also add a fixed amount of usually olomoucine as internal standard to calibrate the readout of the machine. For measuring, we use Liquid Chromatography / Mass Spectrometry (LCMS). The LC part is required to preselect between different metabolites as these can be very similar and would thus not clearly separate in the MS. This also diminishes the risk of ion suppression in the MS. It also removes contaminants. An exemplary time-course might look as follows:

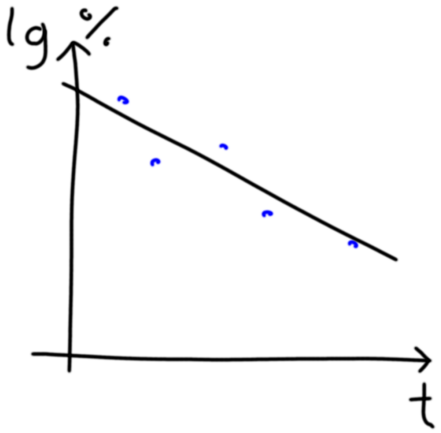

Figure 1: Remaining log percentage of the initial drug concentration over time.

To determine the half-life $t_{1/2}$ we fit a curve to this. Depending on the kind of drug, different half-lifes are preferable: **Cisplatin** is a suicide inhibitor, killing all actively cycling cells. It is only effective in its initial form, after metabolic transformation it doesn't have an effect anymore. Its half-life is extremely short, which is good – otherwise it would kill too many cells and thus be deadly. In contrast, trojan molecules / prodrugs are not active until the trojan group is removed; however, metabolising the prodrug too quickly might cause an unreasonably quick elimination. In some cases,

[3] https://en.wikipedia.org/wiki/Microsome
[4] ibid.

like **abiratrarone**, the actual drug has a very low half-life, whereas the prodrug gets metabolised quickly. This is ideal.

The half-life then can be used to roughly estimate the **bioavailability**, i.e. how much of a drug goes into the blood after the first pass. A very fast degradation hints at a small bioavailability, whereas slower degradation suggests a larger bioavailability.

Another important parameter is the **in-vitro clearance** $[\mu l/(min \cdot mg)]$, which quantifies how fast the drug leaves the system. It is a rate, which is calculated based on the half-life by multiplying the inverse half life with $\ln 2$.

Elucidating which CYP exactly is responsible for degrading a given drug requires lots of work, time, and money. Usually, just the major ones which are most likely will be tested. One can either rerun the experiment and add selective inhibitors of certain CYPs like ketoconazole which primarily inhibits CYP3A4. One can also use recombinant CYP enzymes, which is the best, but also a very expensive method. Thus, the latter is only done if the drug is expected to be really good (e.g. if you want to decide between two out of 2000 really good candidates – so-called "lead compounds").

Another important parameter is the **volume of distribution** $V_d[l]$, also called the "apparent volume of distribution". It is *the theoretical volume [of a human or animal body] that would be necessary to contain the total amount of an administered drug at the same concentration that it is observed in the blood plasma.*[5]. Mathematically, this is the ratio of drug dose divided by the measured plasma concentration of the drug. Therefore, the **dose required to give a certain plasma concentration can be determined** if the V_D for that drug is known[6]. Biologically, V_d quantifies how well a drug is transported into fat and other tissue as opposed to staying in the plasma. Generally, $V_d > 50$ denotes a good fat-solubility. E.g. THC has $V_d = 200$, Vafarin is the opposite with $V_d = 8$ [7]. We can **estimate the volume of distribution in the lab** by putting the drug into a 50/50 mix of water and octanol. After mixing, a MS is run to determine the amount of drug in the octanol. One can also just use blood plasma from yourself.

Protein binding is another important parameter, since blood contains lots of proteins and **these carry the drug to its target.** One should aim for a medium protein binding percentage ($\sim 90\%$ is good). **To estimate *in-vivo* protein binding in the lab**, one uses plates with a membrane through which only the drug can pass. Albumin is placed on the other side of the membrane and after some time, the albumin is quenched in methanol and put into a MS. This gives a percentage which perfectly correlates with the *in-vivo* protein binding percentage.

Finally, the **permeability** of a drug through the intestine is naturally of importance as well. One may use CaCO2 cells since these polarise and thus recapitulate the structure of the real tissue. They also express **transport proteins**, which are potentially important – e.g. drugs can inhibit transport proteins, which would interfere with other medications. The method is not perfect (as the cell line is taken from an intestinal cancer), but works reasonably well.

4 Drug Interactions

We may **predict drug interactions if we know the exact CYPs that are acting upon the respective drugs**. Ideally one uses drugs which only make use of two CYPs (a major and a minor modification is ideal) to avoid too many interactions. However, **metabolites** of a drug may interact via completely different CYPs with other drugs which makes it more complicated (e.g. verapamil).

Enzyme induction in living cells: Usually studied in vivo via proteomics to elucidate how many additional proteins are produced by the cell in response to the drug. This

[5] https://en.wikipedia.org/wiki/Volume_of_distribution
[6] ibid.
[7] ibid.

often happens via binding to a TF, or (rarely) by a direct binding to the DNA. E.g. St Johns Wart, which induces liver cells to produce CYPs 3A4, 2C9, 2C19, and others.

Suicide-inhibition is another, particularly important form of drug interaction, where a substance kills off the entire pool of an enzyme permanently by covalently binding (instead of a competitive reversible binding). E.g. Grapefruit juice.

5 Modelling

ADME is a framework for mathematical modelling based on differential equations. There is also physiology-based PK modelling, for which you can use the numbers measured before. There might be some modelling on "AT13148".