Personalised Medicine and Antibiotics:
Should your DNA decide your dose?

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Introduction

- **Antibiotics remain the major treatment for bacterial infections**
- **Studies with other drugs show they are metabolised at different rates in different individuals, does this also apply to antibiotics??**

Rapid metabolism of antibiotic = efficacy of the drug could be compromised and treatment could fail

OR

Slow metabolism of antibiotic = drug level remains high and increases likelihood of toxicity

Drug Biotransformation Reactions in Humans

Phase I (Functionalization) **Phase II (Conjugation)**

- **The enzymes that perform these reactions are primarily found in the liver and are highly polymorphic, meaning some people have non functional versions of these enzymes.**

Project Aim

Using Human Liver cancer cell lines:

- **Map polymorphisms in genes involved in drug metabolism**
- **Assess the effects of these polymorphisms on metabolism of antibiotics**

Genotyping results

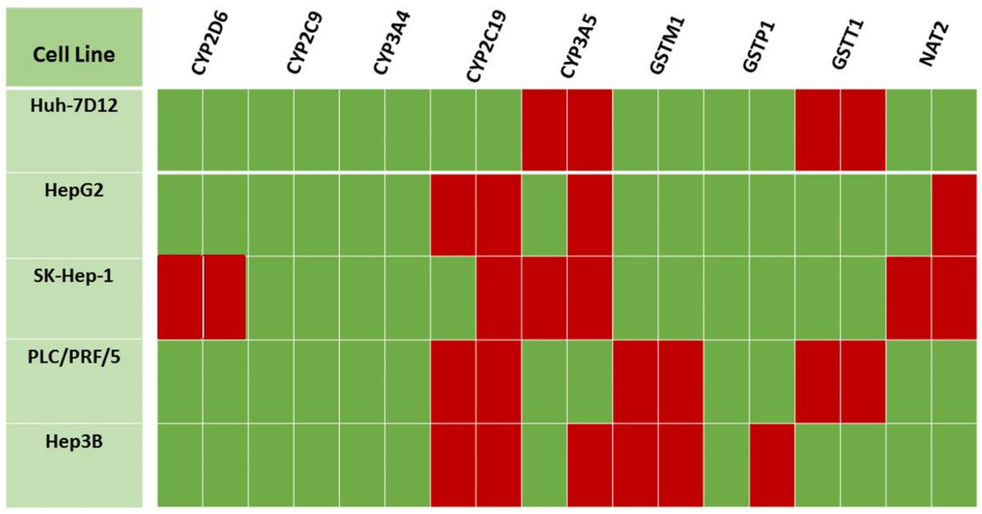


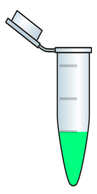
Figure 1. Genotyping using Taqman genotyping assays and conventional PCR-RFLP techniques
Two boxes per enzyme, representing each allelle present:
Green Box = wild type allele, Red box = Mutant allele.
CYP enzymes chosen for genotyping are most polymorphic p450 oxidases involved in phase I metabolism
GST (Glutathione-S-Transferases) and NAT2 enzymes are involved in phase II of drug metabolism

Testing Antibiotic Metabolism in Human Hepatoma cells - Method

Liver cells seeded into 24 well plate at 1 x 10^5 cells per well

Cells left to attach and settle for 36 hrs

Cells washed with PBS and then given a 2µg/ml dose of Amoxicillin



Supernatant removed at Time 0 and 24hrs

Supernatant diluted (doubling dilutions to x8) - 100µl in 96 well plate

100µl of *Staph. Aureus* added to each well (1 x 10^5 /well)
Incubated at 37˚ C for 24 hrs (static)
Read OD 595

- **Increased OD 595 = Reduction in level of active antibiotic present so allowing bacterial growth = liver cells have metabolised the antibiotic**

Amoxicillin Metabolism by Human Hepatoma Cell Lines - Results

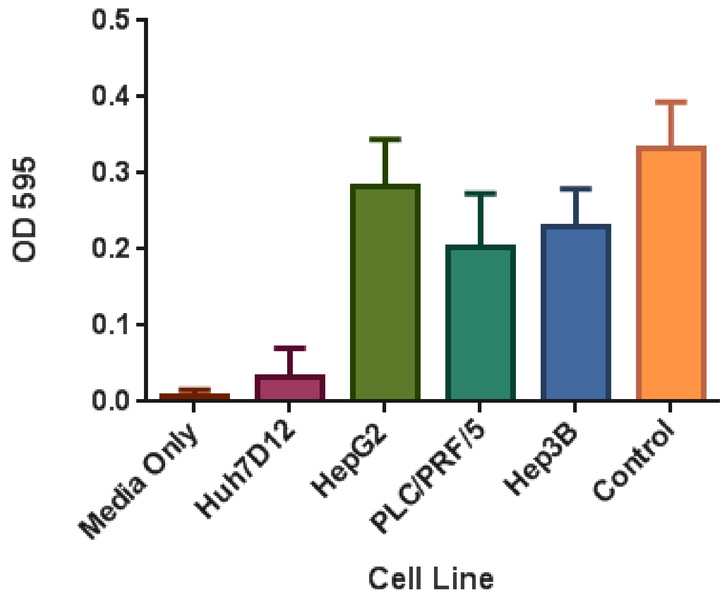


Figure 2. Amoxicillin metabolism by Human hepatoma cell lines.
Media Only control = media plus antibiotic only, no liver cells present, confirms that amoxicillin does not naturally degrade after 24hrs at 37˚ C.
Control = *Staph. Aureus* growth control, no antibiotic present.
Data shown: 24hrs supernatant sample, x8 dilution.

Conclusions

- Human Hepatoma cell lines tested in this work show genetic variation in selected enzymes involved in drug metabolism, with every cell line carrying at least four mutant alleles (Figure 1).
- We have shown that these cell lines metabolise a clinically relevant dose[1] of the antibiotic, Amoxicillin (Figure 2).
- There are differences in the levels of metabolism between the genetically different cell lines. The HepG2 cell line shows significantly higher levels of metabolism of amoxicillin (Figure 2).
- Preissner et al [2] suggests CYP2C19 responsible for metabolism of amoxicillin in humans, however our data indicates that this may not be the case.
- Although Cytochrome p450 class has more than 50 enzymes, just six of them metabolise 90% of drugs. CYP3A4 being one the most significant and most prevalent in the liver. Thus we believe CYP3A4 or CYP3A5 are more likely candidates for the metabolism of amoxicillin.
- We are currently using **CRISPR Gene Editing technology[3]** to perform individual gene knock outs of CYP3A4 , CYP3A5 and CYP2C19 enzymes in the HepG2 cell line. This may allow us to identify the enzyme (s) responsible for the metabolism of amoxicillin.

Acknowledgements

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References

1. Szultka, M et al., Biomed. Chromatogr. 2014; 28: 255-264
2. Preissner et al., J Drug Metab Toxicol 2012, 3:5
3. Charpentier & Doudna 2013 Nature 495, 50-51