A MULTI-LEVEL APPROACH TO INVESTIGATE CYP2D6 DEPENDENT METABOLISM OF CLOMIPHENE

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Introduction

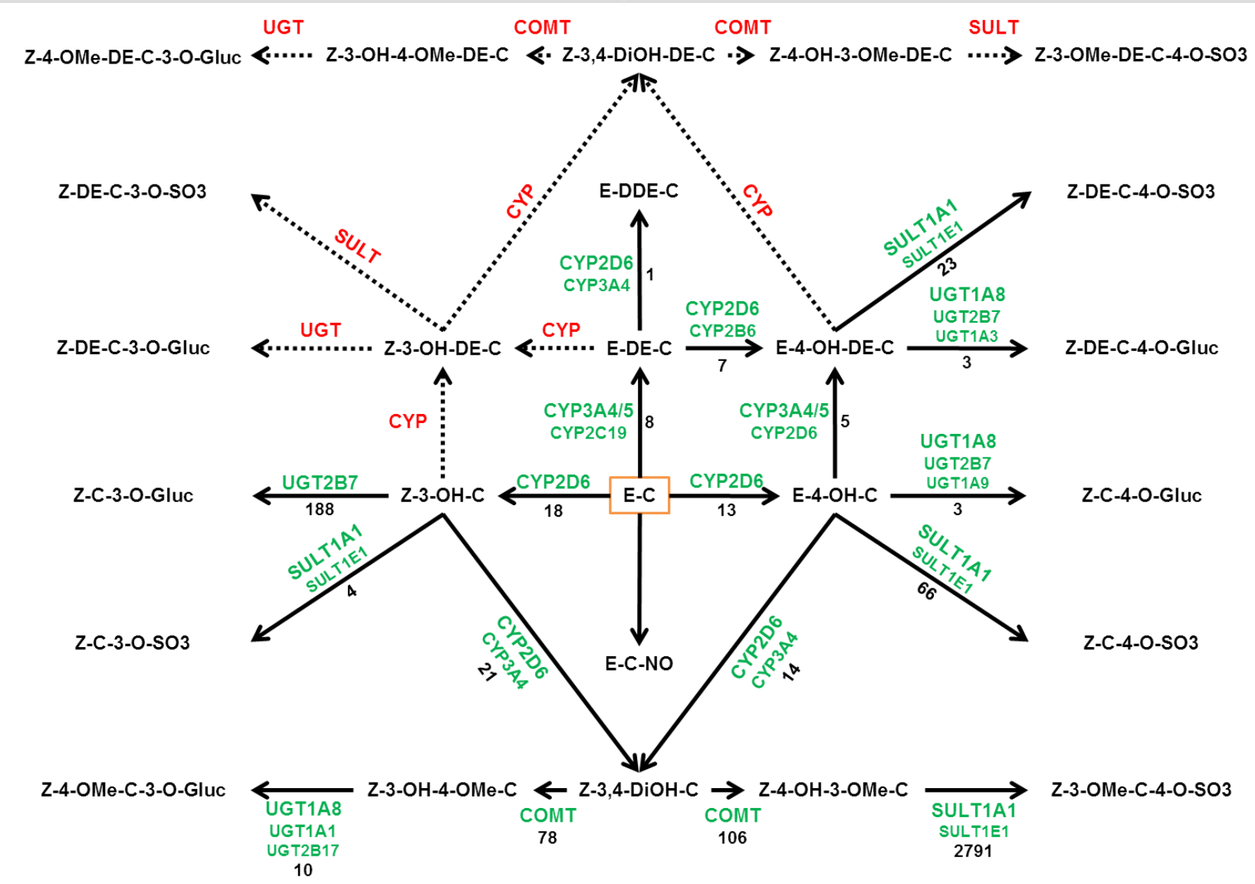
Clomiphene citrate, a selective estrogen receptor modulator, is used as first-line therapy of infertility due to absent or irregular ovulation. However, high inter-individual variability was observed and approximately 25% of the patients do not benefit. (*E*)-clomiphene (*E*-C) is metabolized extensively e.g. via CYP2D6 to the highly potent (*E*)-4-hydroxyclomiphene (*E*-4-OH-C) and (*E*)-4-hydroxy-N-desethylclomiphene (*E*-4-OH-DE-C). Here, we focused on the identification of metabolic pathways involved in the formation and the clearance of these active metabolites.

Methods

- Identification of metabolizing enzymes and determination of the enzyme kinetic parameters via *in vitro* incubations of human liver fractions and recombinant enzyme expression systems.
- Analysis of the cellular clomiphene metabolism in upcyte® hepatocytes.
- In a pharmacokinetic study 20 female healthy volunteers who were stratified according to their CYP2D6 genotype into four groups: poor metabolizer (PM), intermediate metabolizer (IM), extensive metabolizer (EM), and ultra-rapid metabolizer (UM) received a single dose of 100 mg clomiphene citrate po. Plasma samples were collected over 168h and analyzed by LC-MS/MS to generate pharmacokinetic profiles of (*E*)-clomiphene and its metabolites.
- *in silico p*opulation PK-modelling of the metabolic profiles of the pharmacokinetic trial.

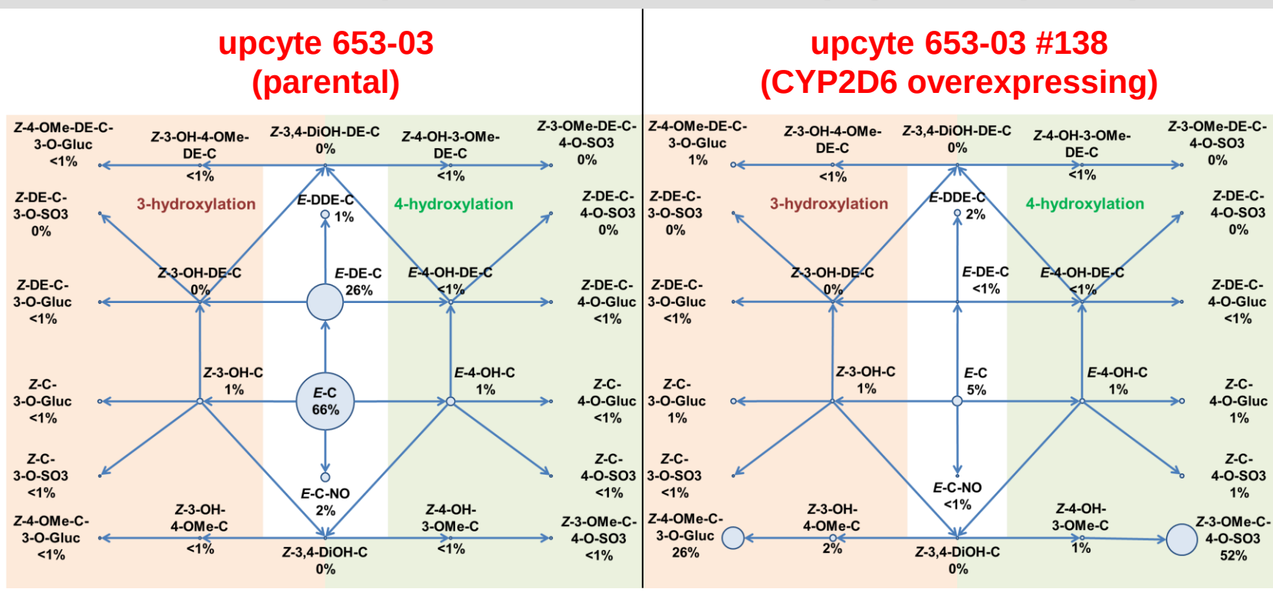
Results

1. *in vitro* Incubation of human liver fractions and recombinant enzymes



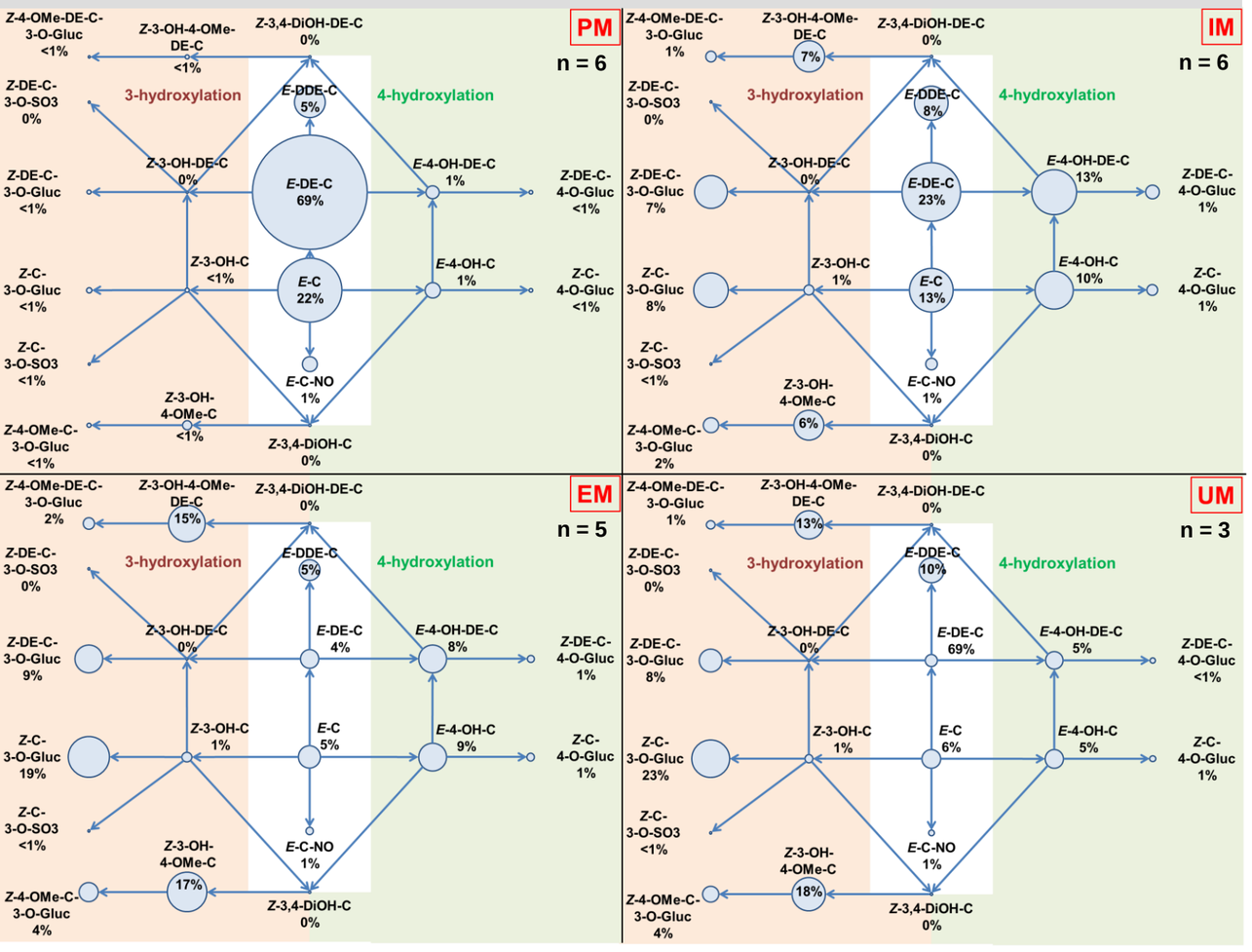
Metabolic scheme of (*E*)-clomiphene (**orange**) combining the metabolic pathways (➔) and the involved enzymes (**green**) identified *in vitro*. The intrinsic clearance in either human liver microsomes or cytosol is given as µl*min^{-1}*mg^{-1} (**black number**). In addition, hypothesized pathways of deethylated metabolites (⇢) and related enzyme families (**red**) are shown.

2. cellular clomiphene metabolism in upcyte® hepatocytes



The cellular metabolism was investigated by incubating upcyte® hepatocytes of a single donor with 1 µM (*E*)-clomiphene. These hepatocytes retain their normal phase 1 and 2 enzyme activities. However, as the parental cells do not show CYP2D6 activity a sub-clone expressing high levels of CYP2D6 was included in the analysis. This change in the enzyme expression pattern resulted in a tremendous shift in the metabolism of *E*-C. The parental cells showed mainly the N-desalkylation of *E*-C whereas the CYP2D6 overexpression led to an almost exclusive accumulation of the hydroxy-methoxyclomiphene conjugates.

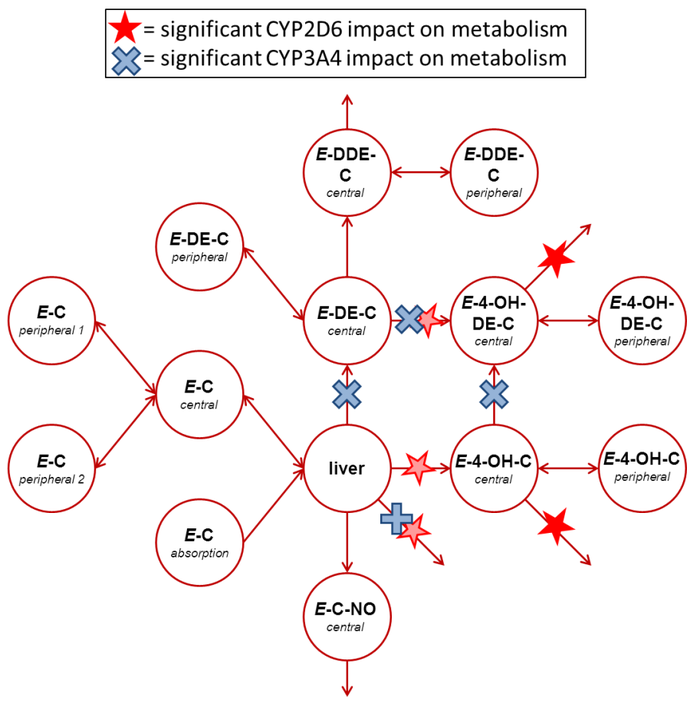
3. voluntary pharmacokinetic study

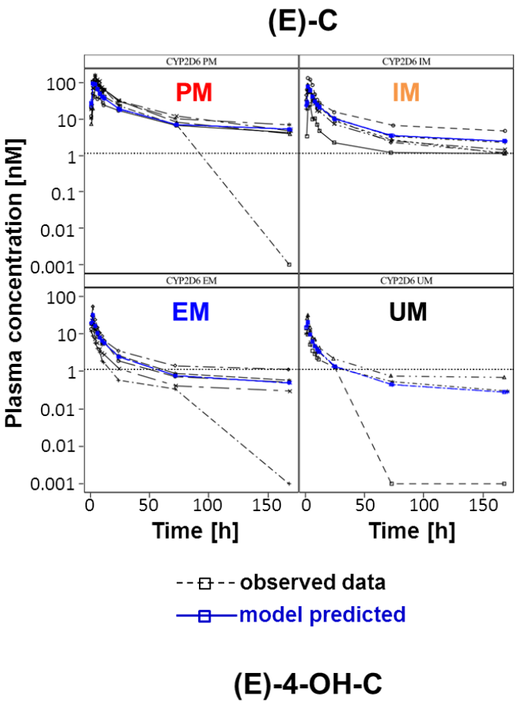


The areas under the concentration time curves (AUC in nM*h) for (*E*)-clomiphene and its metabolites were calculated from plasma samples collected up to 168h after the administration of a single oral dose of 100 mg clomiphene citrate. The areas of the circles for each metabolite represent the absolute amount of AUC. In addition, the percentage of the total AUC is given.

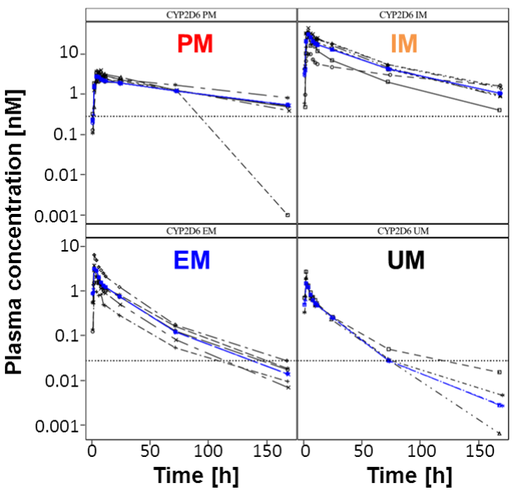
In contrast to the most active metabolites *E*-4-OH-C and *E*-4-OH-DE-C, which showed the highest AUCs in IM subjects, increasing CYP2D6 activity (PM < IM < EM < UM) led to an increase in the AUC of (*Z*)-3-hydroxy-4-methoxy-clomiphene *(Z*-3-OH-4-OMe-C). The latter was proven to be inactive in the estrogen response element (ERE) reporter assay.

4. *in silico* population PK-modeling





In a first *in silico* modeling approach for the bioactivation of clomiphene only a phase 1 metabolite selection of the (E)-isomer was included. However, additional CYP2D6-dependend metabolic reactions (★) for *E*-4-OH-C and *E*-4-OH-DE-C needed to be implemented to perfectly describe the PK of all metabolites included in this model.



Conclusion

This comprehensive analysis revealed a CYP2D6 dependent bioactivation of *E*-Clom to *E*-4-OH-Clom and *E*-4-OH-DE-Clom followed by a CYP2D6 dependent deactivation to dihydroxymetabolites and their respective conjugates. However, the impact of CYP2D6-genotype on patients' outcome needs to be evaluated in a prospective clinical trial.

Acknowledgement

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