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 population PK-modelling of the metabolic profiles of the pharmacokinetic trial.

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

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 metabolite (Z-3-OH-4-Ome-C). The latter was proven to be inactive in the estrogen response element (ERE) reporter assay.

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.

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