

Electrochemical and Enzymatic *In Vitro* Studies on Reactive Drug Metabolites

Synthesis, Characterization and Avoidance

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för avläggande av filosofie doktorsexamen i kemi som, med medgivande av Institutionen för kemi, Göteborgs universitet, kommer att försvaras offentligt onsdagen den 12 maj 2010 kl. 9.15 i sal KB, Kemigården 4, Göteborgs universitet och Chalmers Tekniska Högskola. Avhandlingen kommer stt försvaras på engelska.

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ABSTRACT

During development of pharmaceuticals, it is essential to as early as possible identify and preferably avoid formation of reactive metabolites and intermediates. Reactive metabolites may represent obstacles in the development and use of drugs, due to their potential toxicity. The formation of reactive metabolites often involves oxidation reactions and the cytochrome P450 (CYP) enzymes are the most important enzymes catalyzing oxidative phase I drug metabolism. The present studies were initiated with the aim to chemically mimic phase I drug oxidations, using electrochemistry for characterization and identification of reactive drug metabolites and to avoid metabolic activation by altering the chemical structure of the parent drug.

Three complementary chemical systems were evaluated for their abilities to mimic CYP-catalyzed oxidations. All relevant oxidative reactions were mimicked by at least one of the three systems. The oxidative metabolism of the antimalarial agent amodiaquine was studied in liver microsomes and recombinant enzymes. Electrochemical oxidation was used to characterize metabolic intermediates and enabled the structural determination of an aldehyde metabolite of amodiaquine by NMR spectroscopy. In addition, the bioactivation of the neuroleptic drug haloperidol was studied. Proposed iminium species of haloperidol were observed in the on-line electrochemical oxidation setup. Subsequent trapping with cyanide, both in liver microsomes and in the electrochemical system, strengthened the proposal of iminium intermediate formation in the oxidative metabolism of haloperidol. Further, in the cyanide trapping experiments the presence of the dihydropyridinium species and the absence of the corresponding cyano adduct indicated that an unstable cyano adduct was formed. Trapping of an exocyclic iminium species with cyanide in the electrochemical experiments but not in the liver microsomal incubations implied that this intermediate, obligatory in the electrochemically mediated *N*-dealkylation, may not be formed in the CYP-catalyzed reaction.

Metabolic studies on haloperidol and trifluoperidol, in comparison with their corresponding silicon analogues, were performed in liver microsomes and hepatocytes. The replacement of one single carbon atom by a silicon atom resulted in significant metabolic changes, including the absence of silapyridinium metabolites and glucuronidation on the silanol group.

In conclusion, several different examples of how electrochemistry can be used in studies on reactive metabolites are provided in this thesis. For drug risk assessment, it is important to characterize formed reactive metabolites and if possible, alter chemical design to avoid reactivity.

Keywords: Cytochrome P450, Drug, Electrochemical oxidation, Electrochemistry, Intermediate, Metabolism, Metabolic activation, Mimicry, Reactive Metabolite, Trapping.
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