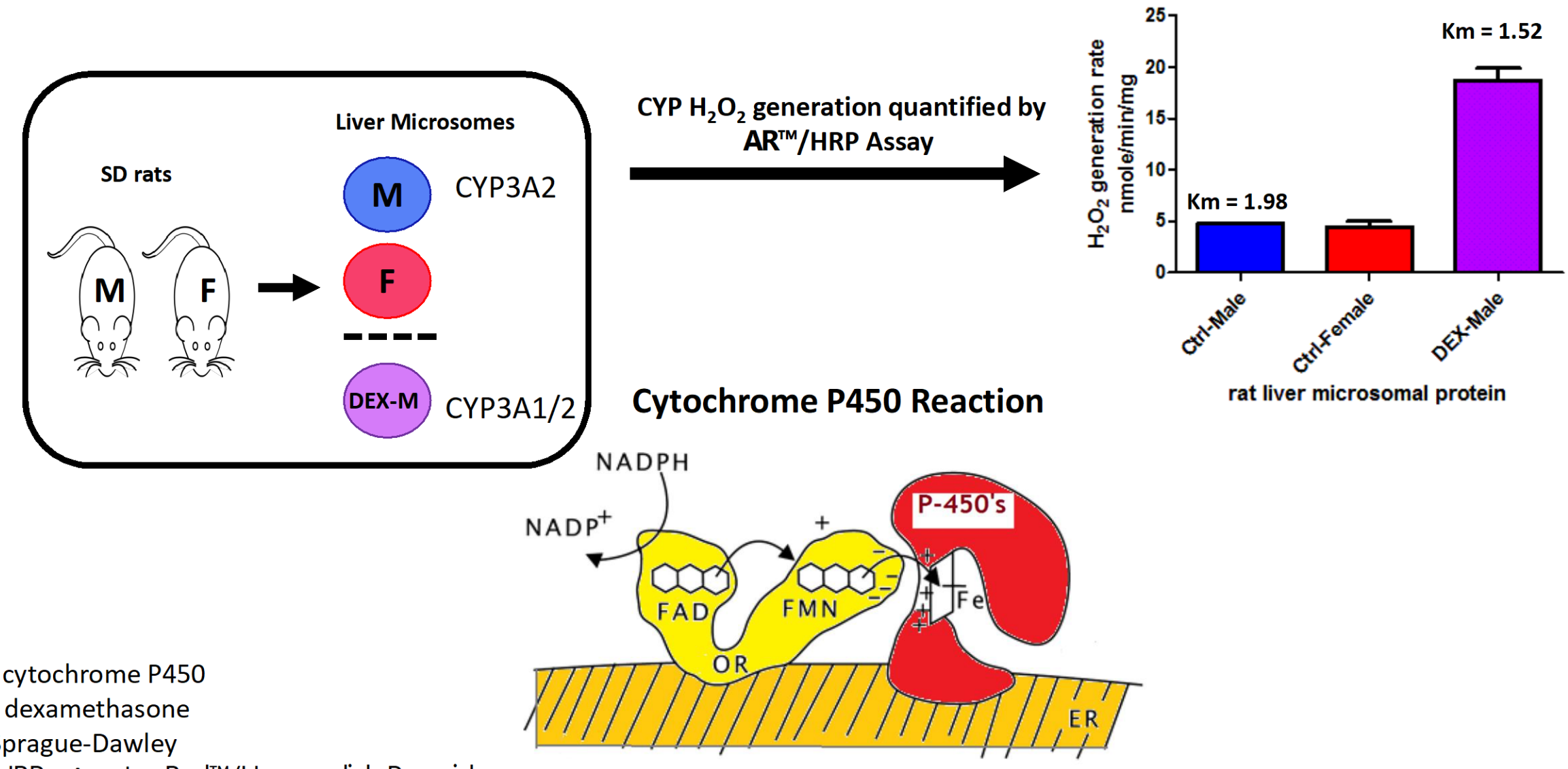
Enzyme Kinetic Parameters for Hydrogen Peroxide Generation (Autoxidation) in P450-Related Microsomal Electron Transport Chains

Jaylen Taylor, Vladimir Mishin, Jeffrey Laskin
Eastern Michigan University and Rutgers, The State University of New Jersey

It is well known that a microsomal electron transport chain with terminal oxidase cytochrome P450 (CYP) enzymes generates hydrogen peroxide. This reaction requires NADPH and oxygen, and can proceed with or without metabolizing substrates for the CYP enzymes. Levels of hydrogen peroxide produced by microsomal electron transport in rat liver microsomes (Sprague-Dawley [SD] rats) depend on multiple factors including the specific set of CYP enzymes expressed in the microsomes. The array of P450 enzymes is significantly different in microsomes from female and male rats and in microsomes from drug treated animals. In the present studies the enzyme kinetic parameters of hydrogen peroxide generation was estimated in different types of microsomes in the presence of NADPH using a highly sensitive method of hydrogen peroxide quantification, the Amplex Red™/Horseradish Peroxidase Assay. Through the application of this method, low background activity of hydrogen peroxide generation was detected in SD rat liver microsomes. Hydrogen peroxide generation rates in microsomes from female and male rats and drug treated (dexamethasone, DEX) rats showed that the CYP 3A enzymes, CYP3A1 and CYP3A2, are the main factors controlling the rate of hydrogen peroxide formation. Interestingly, the Michaelis-Menten constant (Km) is similar in microsomes from male rats and DEX treated rats. This may represent a similar affinity of CYP's enzymes for the NADPH-cytochrome P450-reductase during the formation of hydrogen peroxide. Supported by the SOT Intern Program and NIH R25ES020721.



CYP = cytochrome P450
DEX = dexamethasone
SD = Sprague-Dawley
AR™/HRP = Amplex Red™/Horseradish Peroxidase