H_2O_2 Generation in the Presence of Form Selective Inhibitors of Cytochrome P450 Monooxygenase Activity

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NADPH-cytochrome P450 reductase transfers electrons from NADPH to the heme center of cytochrome P450 (CYP) leading to the reduced form of CYP. The reduced CYP is capable of binding oxygen and the iron-oxygen complex can form different activated oxygen species during several steps generally named NADPH oxidase (uncoupling). In other words, these oxygen intermediates can decay, which causes the release of hydrogen peroxide and/or superoxide anions instead of the production of the desired activated oxygen required for CYP substrate metabolism. 3-(p-Hydroxyphenyl)propionic acid/Horseradish peroxidase (HPPA/HRP) is a fluorescent assay that can be used to measure the rate of hydrogen peroxide production in biological samples. We used this assay to characterize the effects of form selective P450 inhibitors on beta-naphthoflavone (BNF), dexamethasone (DEX), and isoniazid (INH) treated microsomes. Certain CYPs are highly inducible, for example, CYP1A1 and CYP1A2 by BNF, CYP3A1 and CYP3A2 by DEX and CYP2E1 by INH. Therein, our aim is to determine if Alpha-naphthoflavone (ANF), Ketoconazole (KET), and 4-methylpyrazole (4-MP) are the respective inhibitors of H_2O_2 production in these P450s. We observed a weak inhibition of H_2O_2 formation in BNF-induced microsomes by ANF, a strong inhibition of DEX-induced microsomes by KET, and no inhibition of INH-induced microsomes by 4-methylpyrazole. Supported by NIH U54AR055073, P30ES005022, and R25ES020721.