CYP2E1 Mediated H_2O_2 Generation in Nash Reaction Based Assay

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Cytochrome P450 (CYP) is a ubiquitous haemoprotein that is responsible for a large proportion of xenobiotic metabolism and as such is the subject of intense pharmacological and toxicological study to characterize its effects on drug metabolism. Microsomal CYP's, in the presence of NADPH and absence of metabolizable substrates generates partially reduced oxygen species, including superoxide anion via a reaction known as NADPH oxidase activity. Superoxide is readily converted to hydrogen peroxide via a number of enzymes such as the superoxide dismutases. CYP 2E1 is a CYP enzyme that is widely believed to be a exceptionally active generator of H_2O_2. The relative contribution of individual CYP is difficult to quantify due to many technical difficulties. Within this study we attempted to characterize H_2O_2 production by CYP 2E1 in various microsomal samples and use some CYP form-selective inhibitors to compare levels of H_2O_2 generation. A Nash reagent-based assay was used to quantify the amount of H_2O_2 generated by a microsomal sample by using the catalytic activity of catalase to react with H_2O_2 in the presence of methanol to generate formaldehyde. This mixture was then incubated with the Nash reagent which utilizes the Hantzsch reaction to produce diacteyldihydrolutidine (DDL), a fluorescent molecule that could be measured in a microplate reader. In the calibration samples, the amount of DDL was directly proportional to the amount of formaldehyde which in turn was directly proportional to the amount of H_2O_2 in the sample allowing for quantification of H_2O_2. The results show that 2E1-enriched microsomes do not generate as microsomes enriched with other cytochrome P450 enzymes. Our data suggest that CYP 2E1 is in fact not as powerful an H_2O_2 generator as previously believed. More study into the effects of inhibitors such as 4-methylpyrazol on various microsomal samples induced by xenobiotics would further elucidate the relative contribution of 2E1 in H_2O_2 generation. Supported by NIH P30ES005022 and R25ES020721.