Poster Session 3 – Drug Metabolism

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Phenylalanine 4-monooxygenase: a susceptibility factor for neurological diseases

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The metabolism of the drug, S-carboxymethyl-L-cysteine (SCMC) is known to exhibit pharmacogenetic variation in the production of S-oxide metabolites and this polymorphism has been under active investigation as a susceptibility factor for Parkinson's and motor neurone diseases (Steventon et a, 2001). To date, the identity of the enzyme responsible for this S-oxidation biotransformation is unknown.

Based on the observations that the cytosolic enzyme phenylalanine 4-monooxygenase (PAM) has the ability to S-oxidise L-methionine and S-methyl-L-cysteine (Kaufman & Mason 1982), this enzyme has been examined for its potential to mediate the S-oxidation of SCMC. PAM is a tetrameric allosteric protein, which can be activated via a number of mechanisms involving the binding of the activator to the N-terminal regulatory region of the enzyme. However, three of these activation processes result in a change of substrate specificity to include the above sulphur containing amino acids. Work in the Molecular and Cellular Toxicology Research Laboratory has now shown that activation of phenylalanine 4-monooxygenase with either N-ethylmaleimide, reduced glutathione or lysophosphatidylcholine produced a rat hepatic cytosolic fraction with the capacity to S-oxidise SCMC (Table 1). Rat cytosolic fractions were incubated with 1 mM SCMC according to the method of Shiman (1987). The acid-precipitated supernatants were subjected to strong cation exchange solid phase extraction. The eluants were derivatised with OPA/2ME and analysed by HPLC with fluorescence detection.

Table 1 Phenylalanine 4-monooxygenase activity in rat hepatic cytosol fractions

Pre-incubation treatment	PAM – (Def & Phe)	PAM + 1 mM Def	PAM + 1mM Phe
Control	ND	ND	ND
N-ethylmaleimide (1mM)	30.0 ± 1.5	ND	ND
GSH (1mM)	26.5 ± 1.4	ND	ND
Lysophosphatidylcholine (1mM)	33.7 ± 1.7	ND	ND

Activity is presented as nmol SCMC S-oxides per min per mg and are means ± s.d. of 6 determinations. ND, < 0.005 nmol SCMC S-oxides formed per min per mg

The rat hepatic cytosols required both Fe^{2+} for activity and tetrahydrobiopterin as a cofactor. The conversion of SCMC to SCMC *S*-and *R*- S-oxide metabolites was inhibited by deferoxamine (Def) and phenylalanine (Phe). These results indicate that the identity of the enzyme responsible for the S-oxidation of SCMC is phenylalanine 4-monooxygenase. The nature of the molecular defects in the enzyme that result in the S-oxidation polymorphism is currently under investigation.

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