World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:8, No:05, 2014

The Role of High Performance Liquid Chromatography in Identification of Rat Liver Microsomes Responsible for the in vitro Metabolite Formation of Dipyrone

Authors : Salem Abdalla

Abstract : Objective: Dipyrone is a widely used, well tolerated analgesic drug which, however, is compromised by agranulocytosis as an adverse effect. Subsequent to no enzymatic hydrolysis, the primary metabolic step is N-demethylation of 4-methylaminoantipyrine (4-MAA) to 4-aminoantipyrine (4-AA). The aim of the present study was to identify the cytochrome P-450 enzyme (CYP) mediating this reaction. Methods: We identified the relevant CYP using virus expressed isolated rat liver microsomes with chemical inhibition studies. The substrate of 4-methylaminantipyrine was employed at six different concentrations (25, 50, 100, 400, 800, and 1200 µmol/l) with varying concentrations of selective inhibitors of CYP1A2 (furafylline, fluvoxamine), CYP3A4 (ketoconazole), CYP2A6 (coumarin), CYP2D6 (quinidine), CYP2C19 (omeprazole, fluvoxamine, tranylcypromine), CYP2C9 (sulfaphenazole), and CYP1A1 (alpha-naphthoflavone). 4-MAA and 4-AA were analyzed by HPLC, and enzyme kinetic parameters (Km and Vmax) were determined by regression (Sigma plot 9.0). Results: The N-demethylation of 4-MAA by microsomes prepared from baculovirus-expressing human CYP was pronounced with CYP2C19. Intrinsic clearances of the most active enzymes were 0.092, 0.027, and 0.026 for the CYP enzymes 2C19, 2D6, and 1A2, respectively. Metabolism by rat liver microsomes was strongly inhibited by omeprazole (IC50 of 0.05). Conclusion: The enzyme CYP2C19 apparently has an important role in N-demethylation of 4-methylaminoantipyrine which should be further analyzed in clinical studies and which may also be interesting concerning the agranulocytosis.

Keywords : dipyrone, 4-methylaminoantipyrine (4-MAA), 4- aminoantipyrine (4-AA), metabolism, human CYP2C19
Conference Title : ICBB 2014 : International Conference on Bioinformatics and Biomedicine
Conference Location : Istanbul, Türkiye
Conference Dates : May 22-23, 2014