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Identification of human cytochrome P450 isozymes responsible for the in-vitro oxidative demethylation of 4-methylaminoantipyrine

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Abstract: The aim of this study is to identify human cytochrome P-450 enzyme (CYP) mediating the oxidative N-demethylation of 4-methylaminoantipyrine (4-MAA) to 4-amino-antipyrine (4-AA). The contribution of human CYP to the metabolism of 4-MAA to 4-AA in human was investigated by using virus expressed human CYP, human liver microsomes and rat liver microsomes with chemical inhibition studies. The substrate of 4-methylaminantipyrine was employed at five different concentrations (12.5, 23, 46, 115 and 230 µmol/l) with varying concentrations of selective inhibitors of CYP (CYP1A2), (CYP3A4), (CYP2C8), (CYP2A6), (CYP2D6), (CYP2C19) and (CYP1A1). 4-MAA and 4-AA were analyzed by HPLC and enzyme kinetic parameters (K_m and V_{max}) were calculated from the concentration data. The transformation of 4-methylaminoantipyrine to 4-aminoantipyrine by microsomes prepared from baculovirus-expressed human CYP was pronounced with CYP2C19. Metabolism of 4-methylaminoantipyrine by human liver microsomes and rat liver microsomes was strongly inhibited by tranylcypromine, fluvoxamine and omeprazole inhibition was observed with other CYP selective inhibitors. 4-methyl-aminoantipyrine was also evaluated as a CYP substrate in rat liver microsomes. No significant inhibition of CYP1A2, CYP1A1, CYP3A4, CYP2C9, CYP2D6, CYP2A6 and CYP2E1 was observed in experiments (IC_{50} > 269.14 µM) but IC_{50} for CYP2C19 was 68.48 µM. In conclusion, the enzyme CYP2C19 apparently has an important role in N-demethylation of 4- methylaminoantipyrine.

Keywords: Metamizole, 4-methylaminoantipyrine, 4-aminoantipyrine (4-AA), metabolism, CYP2C19.

Introduction

Metamizole (sodium [N-(1, 5-dimethyl-3-oxo-2- phenylpyrazolin - 4 - yl) - N-methyl-amine] methaneulphonate monohydrate) is a pyrazoline derivative available in oral and parenteral forms acting as inhibitor of cyclo-oxygenases. It has also been used as a weak nonsteroidal anti-inflammatory agent (1) as well as a potent analgesic and antipyretic drugs in many countries for more than 60 years. Oral doses of 0.5 to 1 µg. Pereira and colleagues (2) have been effective in treating fever. Repeated doses (up to 4 times daily) can be administered, the maximum recommended dose is 3 to 4 µg daily (3). Metamizole has been associated with fatal agranulocytosis and was withdrawn from the US market in 1979 (1). The complex metabolism of metamizole has been the subject of many in-vivo studies (4). However, the specific CYP catalyzing the formation of the active metabolite 4-AA is still not known. The biotransformation pathway of metamizole (Figure 1) is well established (4). It is non enzymatically hydrolyzed in the gastric juice to the active moiety 4-methylaminoantipyrine4-omes: CYP, cytochrome P-450: HPLC, high performance liquid chromatographic.

Materials and methods

Chemicals: All chemicals and reagents were of analytical grade unless stated otherwise. HPLC-grade acetonitrile and methanol were obtained from J. T. Baker (Mallinckrodt Baker, Holland), the other chemicals and reagents were obtained from following sources: amino antipyrine, ketoconazole, alpha naphtoflavone, omeprazole and sulpha-phenazole were purchased from Sigma chemical (Steinheim, Germany) while coumarin and quinidine were obtained from (Fluka Steinheim, Germany). NADPH was purchased from Roche (Mannheim, Germany) and the internal standard (4-dimethylamino-antipyrine, 4-DMA$_A$) was ordered from Sigma chemical (Steinheim, Germany). Potassium dihydrogen phosphate was purchased from Merck (Darmstadt, Germany).

Microsomes and human P-450 isoforms. Baculovirus-derived microsomes expressing human P-450 CYP3A4/OR (Cat. No. P207, Lot 67), CYP3A5/OR (Cat. No. P235, Lot 21), CYP3A7/OR (Cat. No. P237, Lot 09), CYP1A1/OR (Cat. No. P211, Lot 22), CYP1A2/OR (Cat. No. P203, Lot 28), CYP2C9/OR (Cat. No. P242, Lot 03), CYP2C8/OR (Cat. No. P252, Lot 10), CYP2C19/OR (Cat. No. P219, Lot 19), CYP2D6/OR (Cat. No. P217, Lot 43), CYP2E1/OR (Cat. No. P206, Lot 19), CYP2A6/OR (Cat. No. P254, Lot 07) were all obtained from Gentest (Frankfurt, Main, Germany).

Preparation of microsomes. Human hepatic microsomes were prepared by fractionation as described previously (5). Approximately 8 gm of liver per experiment was allowed to thaw at room temperature in homogenization buffer (Tris 20 mM, Na-EDTA 5 mM, sucrose 254 mM pH 7.4 in ice bath). The suspension was centrifuged at 9,000 g for 30 min and the resulting supernatant was further centrifuged at 105,000 g for 60 min in an ultracentrifuge. The microsomal pellets were suspended in 250 mM sodium/potassium phosphate buffer (pH 7.4) containing 5 mM EDTA and 30% glycerol (v/v). They were stored in aliquots at -80 until use. Microsomal protein concent-ration was determined by the method of (6) with bovine serum albumin as a reference standard. The rat liver microsomes were prepared following the same procedures described for human liver microsomes and protein concentration was determined using the BCA method (Pierce chemical, Rocford, IL) (7).

Incubation conditions

A. General protocol
In Eppendorf reaction vessels, 250 µl of methylaminoantipyrine solution in methanol, representing one of five concentration levels (HPLC, Instrumentation and chromatographic conditions). The HPLC is very suitable to study the formation of metamizole metabolites in human liver microsomes (8, 9). The HPLC system consisted of an L-600A pump (Merck, Hitachi Tokyo, Japan) and 655A-40 autosampler (Merck, Hitachi, Tokyo, Japan). The system was equipped with LiChrospher 100 RP-8e select column 5 µm (Merck, Darmstadt, Germany) preceded by a pre-column (100 Diol, 5 µM). The mobile phase consisted of 80% of 50 mM sodium phosphate buffer (pH 6.0), 15% acetonitrile and 5% methanol. The flow rate was 1.0 ml/min.

The absorbance was measured at 254 nm, linked to computer data system with an ultraviolet (UV) detector (655 A Merck Hitachi Tokyo, Japan). The injection volume in these analyses was 40 µl, and the retention times of 4-methylaminoantipyrine (4-MAA), 4-aminoantipyrine (4-AA), and 4-dimethyl-aminoantipyrine (4-DMAA) were 7.05, 5.37 and 9.38 minutes, respectively.

HPLC-method: sensitivity, precision, selec-tivity (separation of inhibitors). Incubation of 4-methylaminoantipyrine with microsomes resulted in the formation of the 4-aminoantip-yrine as the only metabolite of 4-methyla-minantipyrine.

Metabolism of 4-methylaminoantipyrine by rat liver microsomes (RLM). Liver tissue from two animals (liver A and B) was available. A mean V_{max} of 58.5 pmol/min was estimated for two samples together with a K_m of 20.9 µmol/l (Table 3). The metabolism of 4-methylaminoantipyrine was strongly inhibited by omeprazole (92% inhibition) and to a lesser degree by ketoconazole (37% inhibition) and but no inhibition was recognized with alpha naphthoflavone, coumarin, quinidine, sulphaphenazole (Table 1).

Table 1:

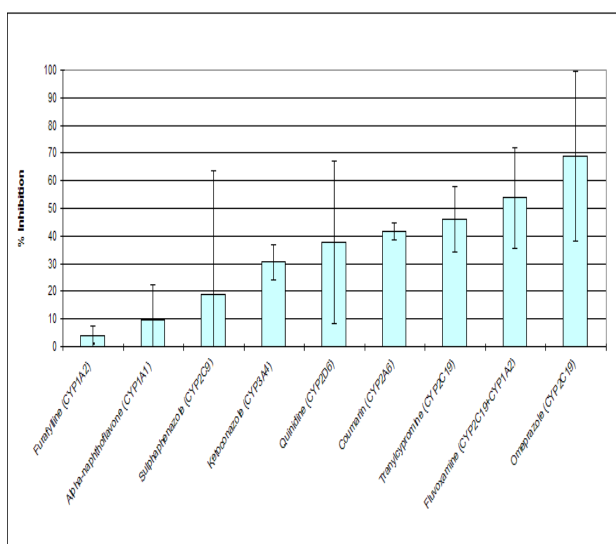


Table 2:

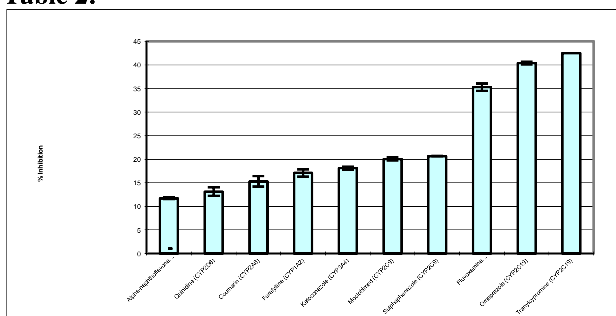
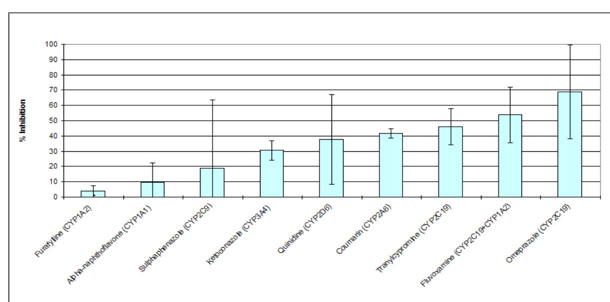


Table 3:



Metabolism of 4-methylaminoantipyrine by human liver microsomes (HLM). With human liver microsomes, a lower maximum transformation rate of 22.5 pmol/l and a similar K_m value of 18.20 µmol/l was obtained than with rat material (Table 2B).

Inhibition metabolism of 4-methylaminoantipyrine, HLM with inhibitors. Inhibitory effect on 4-methylaminoantipyrine N-demethylation was seen for tranylcypromine, fluvoxamine eprazole 1, no relevant inhibition was observed in incubations with ketoconazole, furafylline, sulphaphenazole, alphanaphthoflavone, and coumarin and moclobime3e metabolism of 4-methyl-aminoantipyrine was inhibited by 42.569, 40.4346d 354 by 100 the CYP2C19 inhibitors omeprazole, tranylcypromine and omeprazole an= (0.159.28, 0.061289, and 0.85 mM/l) reactivey with IC_{50} values of 0.2168, .48 0.0895.2 and 1.1711808tivey. The IC values seen with alpha-naphthoflavone, quinidine, sluaphaphenazole, ketoconazole, furafylline, and coumarin and moclobemid we (4.307380, 16.927939, 562.87, 24 561.149 and 5 269.14 02881.00, 1.13, 7.01, 14380 and 06 reti which suggested that CYP2C19 was responsible, primary, for the metabolism of 4-methylaminoantipyrine.

Metabolism of metylaminoantipyrine by virus expressed human P450 isoforms. 4-methylaminoantipyrine was converted into 4-aminoantipyrine by all specimens tested especially at more elevated substrate concentration but considerable formation rates above 75 pmol/min were seen.

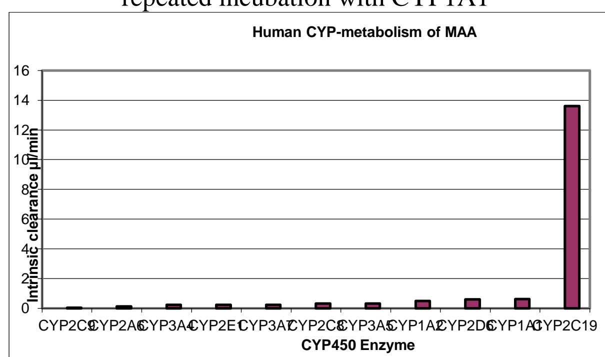
Discussion

Shimada, the major from being CYP2C9 followed by CYP2C19 and CYP2C8 (Stormer et al., 2000) C Goldstein 1994). Our results from the inhibition study indicated the omeprazole, fluvoxamine and tranylcypromine are highly potent selective inhibitors of CYP2C19. Also in other studies as ratio (10, 11) have been high affinity site of 4-

metylaminoantipyrine demethylation and with coumarin (Ki = 46.18 μM) at the low affinity site of 4-metylaminoantipyrine demethylation, respectively. Quinidine had relatively small effect on the metabolite formation rate at the low affinity and very weak affinity was alpha naphthoflavone (Ki = 4268100, Table 1).

The overall results from human liver microsomes incubation experiments with inhibitors and without inhibitors. The V_{max} and K_m parameters studies indicated the CYP2C19 was responsible primarily for the metabolism of 4-methylaminantipyrine. This was evidenced by the ability of CYP2C19 to catalyze the reactions comprising the metabolic pathway of 4-methylaminatipyrine. The strong Inhibition observed with human liver microsomes when tranylcypromine, omeprazole and fluvoxamine were used as inhibitors, Figure 3.

Figure 4: CYP1A2 is the low affinity site and after repeated incubation with CYP1A1



CYP (CYP1A2, CYP1A1, CYP2C19, CYP2A6, CYP2D6, CYP3A4, CYP3A5, CYP3A7, CYP2C8, CYP2C9 and CYP2E1

To determine the CYP reaction phenotype of 4-methylamino-antipyrine microsomes expr-essing individual recombinant human were incubated with different five concentrations from 4-methylaminoantipyrine. Under these conditions the highest formation rate of 4-aminoantipyrine was observed with CYP2C19 (High affinity site with a K_m of (79.69 µml/l). CYP2C19 had the highest catalytic efficiency (intrinsic clearance, V_{max}/ K_m) (± S.D.) of measurements CYP1A2 and CYP2C19 the CYP2C19 is high affinity site a similar effect was observed in the all incubation in the present studies. In conclusion, the data presented here supported the results obtained from clinical studies that CYP2C19 is clinically important enzyme responsible of the metabolism of a number of therapeutic agents (12) and the effect of CYP2C19 in metabolism of methylamino antipyrine. A similar effect was observed for CYP2C19 with S-mephenytoin ratio (12).

References

1. Bonkowsky JL, Frazer JK, Buchi KF and Byington CL. Metamizole use by Latino immigrants: a common and potentially harmful home remedy. Pediatrics. 2002, 109:e98.
2. Pereira PC, Barraviera B, Marcondes J, Leite CV, Meira DA, Inoue T and Morceli J. Progressive subacute paracoccidioidomycosis, treatment of a patient with amphotericin B and parenteral feeding. Rev Inst Med Trop Sao Paulo. 1985, 27: 268-273.
3. Sadusk JF. Planning in the food and drug administration for regulation of prescription drug advertising. Curr Ther Res Clin Exp. 1965, 18: 332-336.
4. Levy M. Pharmacokinetics of metamizol metabolites. Agents Actions. 1986, 19S: 199-204.
5. Nelson AC, Huang W and Moody DE. Variables in human liver microsome preparation: impact on the kinetics of l-alpha-acetylmethadol (LAAM) n-demethylation and dextromethorphan O-demethylation. Drug Metab Dispos. 2001, 29: 319-325.
6. Huskey SW, Dean DC, Miller RR, Rasmusson GH and Chiu SH. Identification of human cytochrome P450 isozymes responsible for the in vitro oxidative metabolism of finasteride. Drug Metab Dispos. 1995, 23: 1126-1135.
7. Tisi DK, Emard JJ and Koski KG. Total protein concentration in human amniotic fluid is negatively associated with infant birth weight. J Nutr. 2004, 134: 1754-1758.
8. Asmardi G and Jamali F. High-performance liquid chromatography of dipyrone and its active metabolite in biological fluids. J Chromatogr. 1983, 277: 183-189.
9. Geisslinger G, Bocker R and Levy M. High-performance liquid chromatographic analysis of dipyrone metabolites to study their formation in human liver microsomes. Pharm Res. 1996, 13: 1272-1275.
10. Salsali M, Holt A and Baker GB. Inhibitory effects of the monoamine oxidase inhibitor tranylcypromine on the cytochrome P450 enzymes CYP2C19, CYP2C9, and CYP2D6. Cell Mol Neurobiol. 2004, 24: 63-76.
11. Hemeryck A and Belpaire FM. Selective serotonin reuptake inhibitors and cytochrome P-450 mediated drug-drug interactions: an update. Curr Drug Metab. 2002, 3: 13-37.
12. Blaisdell J, Mohrenweiser H, Jackson J, Ferguson S, Coulter S, Chanas B, Xi T, Ghanayem B and Goldstein JA. Identification and functional characterization of new potentially defective alleles of human CYP2C19. Pharmacogenetics. 2002, 12: 703-711.
13. Brune K and Otterness I (1988) In vivo and in vitro assessment of non-steroidal anti-inflammatory drugs. Baillieres Clin Rheumatol 2:295-307.
14. Cheng Y and Prusoff WH (1973) Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. Biochem Pharmacol 22:3099-3108.
15. Flusser D, Zylber-Katz E, Granit L and Levy M (1988) Influence of food on the pharmacokinetics of dipyrone. Eur J Clin Pharmacol 34:105-107.
16. Levy M, Zylber-Katz E and Rosenkranz B (1995) Clinical pharmacokinetics of dipyrone and its metabolites. Clin Pharmacokinet 28:216-234.
17. Naritomi Y, Terashita S, Kagayama A and Sugiyama Y (2003) Utility of hepatocytes in predicting drug metabolism: comparison of hepatic intrinsic clearance in rats and humans in vivo and in vitro. Drug Metab Dispos 31:580-588.

18. Vlahov V, Badian M, Verho M and Bacracheva N (1990) Pharmacokinetics of metamizol metabolites in healthy subjects after a single oral dose of metamizol sodium. Eur J Clin Pharmacol 38:61-65.
19. Volz M and Kellner HM (1980) Kinetics and metabolism of pyrazolones (propyphenazone, aminopyrine and dipyrone). Br J Clin Pharmacol 10 Suppl 2:299S-308S.
20. Wang B, Sanchez RI, Franklin RB, Evans DC and Huskey SE (2004) The involvement of CYP3A4 and CYP2C9 in the metabolism of 17 alpha-ethinylestradiol. Drug Metab Dispos 32:1209-1212.