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Research Article

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Study on the Relationship between Genetic Polymorphisms of Cytochrome P450 and effects of Dipyrone in dental pain

[1]Salem O. Ali Abdalla*, [2]Thabet H. Nagah, [3]Ashraf F. Alzalitni and [4]Fathiya Asteal

[1]Al-tahady University, Faculty of Dentistry, Libya
[2]Higher Institute for Medical Occupation Tripoli Libya
[3]Tripoli University, Faculty of Dentistry, Libya
[4]Sirt University, Medicine Technology Faculty, Libya

ABSTRACT

Dipyrone is a non-steroidal anti-inflammatory drug (NSAID), commonly used in the past as a powerful painkiller and fever reducer. It is better known under the brand names Neo-Melubrina®,Analgin®, and Novalgin®. Because of the risk of serious adverse effects, its use is justified only in serious situations where no alternative is available or suitable. Dipyrone is a widely used well tolerated analgesic drug which is how-ever compromised by agranulocytosis as adverse effect. Subsequent to no enzymatic hydrolysis, primary metabolic step is N-demethylation of 4-meth-ylaminoantipyrine (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. 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,CYP2C9 (sulphaphenazole) and CYP1A1 (alpha -naphthoflavone). 4-MAA and 4-AA were analyzed by HPLC, and enzyme kinetic parameters (Km and Vmax) were determined by regression (Sig-ma plot 9.0. The N-demethylation of 4-MAA by microsomes prepared from baculovirus-expressing human CYP was pronounced with CYP2C19. Intrinsic clearance 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). 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. And the Dipyrone is best dental pain because inhibitor the COX in brine

Key words: Dipyrone, 4-methylaminoantipyrine (4-MAA), 4- aminoantipyrine (4-AA), metabolism, Enzyme CYP2C19.

INTRODUCTION

Dipyrone, chemically [(2,3-dihydro-1,5 dimethyl-3-oxo-2-phenyl-1H-pyrazol-4-yl) me-thylamino] methanesulphonic acid, is an effective and widely used drug in several countries because of its analgesic and antipyretic properties1-(Agundez et al., 1994). Dipyrone was first synthesized by the German company Hoechst AG in 1920, and its mass production started in 1922. Dipyrone is a pyrazoline derivative available in oral and parenteral forms acting as inhibitor of cyclooxygenases. It has been used as a no steroidal anti-inflammatory agent 2-

(Bonkowsky et al., 2002) as well as a potent analgesic and antipyretic drug in many countries for more than 60 years. Oral doses of 0.5 to 1 g 3-(Pereira et al., 1985) are effective in treating fever. Repeated doses (up to 4 times daily) can be administered, the maximum recommended dose is 3 to 4 g daily (4-Sadusk, 1965). Dypirone is given orally as capsules or at tablets. Sometimes, users of Dipyrone prefer to swallow the contents of an ampoule for parenteral administration, because they believe that the analgesic effect is quicker than with the usual solid oral forms 5-(Artaza et al., 2002). However, Dipyrone has been associated with fatal agranulocytosis andwas withdrawn from the US market in 1979 (6-Bonkowsky et al., 2002). The complex metabolism of Dipyrone has been the subject of many in-vivo studies (7-Levy et al., 1995). In the pharmacokinetics of Dipyrone, the

specific CYP catalyzing the formation of the primary metabolic step to the active metabolite 4-aminoantipyrine (4-AA) is still not known. The biotransformation pathway of Dipyrone(8-Levy, 1986) and (9-Artaza et al., 2002) is well established. It is nonenzymatically dealkylated in the gastric juice to the active moiety 4-methylaminoantipyrine (4-MAA) (10-Ergun et al., 2004; 11-Vlahov et al., 1990). 4-MAA undergoes demethylation in the liver to 4-aminoantipyrine (4-AA) (12Brune and Otterness, 1988;13-Flusser et al., 1988). AA undergoes further phase-II biotransformation to acetyl-aminoanipyrine (AAA) by the polymorphic N-acetyltransferase (Fig. 1). Dipyrone was developed in times where specific CYP enzymes were not yet known but knowledge of the relevant specific CYP enzymes may help in predicting drug- drug interactions. It may also help in elucidating the relevant bioactivation reactions which in some persons result in agranulocytosis.

CYP2C19 genetic variability

CYP2C19 is a clinically important enzyme which metabolizes a wide variety of drugs, such as the anticonvulsant mephenytoin 14(Wilkinson et al., 1989). Anti-ulcer drugs such as omeprazole (15-Andersson et al., 1992), certain antidepressants (16-Sindrup et al., 1993a) and (17-Baumann et al., 1986), the anti malarial proguanil (18-Ward et al., 1991) and the anxiolytic drugs diazepam. It is also partially responsible for the metabolism of a number of other drugs, such as the β-blocker propranolol metabolism of these drugs in-vivo is polymorphic in humans. Individuals can be characterized as extensive metabolizers or poor metabolizers of drugs metabolized by CYP2C19 in population studies. Poor metabolizers represent 2-5 % of Caucasians, 13- 23 % of Asian populations and as many as 38- 79 % of individuals of some of the islands of Polynesia and Micronesia (19-Kaneko et al., 1999). African population have been studied less extensively, but the poor metabolizer trait has been reported to be approximately 40% in African- Americans from mid- Tennessee (Edeki et al., 1996), Africans from Zimbabwe and Nigerians (20-Daniel and Edeki, 1996). Poor metabolizers can experience undesirable side- effects, such as prolonged sedation and unconsciousness after administration of diazepam. This can be a particular problem in Asian patients where the poor metabolizer phenotype is frequent. In contrast, omeprazole has been reported to produce a greater cure rate for gastric ulcers and accompanying Helicobacter pylori infections in CYP2C19 poor metabolizers than in extensive metabolizers because blood levels are higher in these individuals (21-Goldstein, 2001).For CYP2C19, 16 allelic variants have been identified.CYP2C19*2 and CYP2C19*3 are important, since the CYP2C19*2 allelic variant accounted for 75– 83 % of the poor metabolizer (PM) phenotypes in Caucasian (22-De Morais et al., 1994) and Asian populations (de Morais et al., 1994). Furthermore, CYP2C19*2 and *3 combined, accounted for 100 % of PM phenotypes in a study of a Japanese population (23-Ibeanu et al., 1998).CYP2C19*2 variant causes a 681G>A nucleotide change in exon 5 that creates a splicing defect, and CYP2C19*3 is a 636G>A change that generates a stop codon in exon 4. The distribution (24-De Morais et al.,25- 1994; de Morais et al., 1994), of these allelic variants within different ethnic groups varies. For instance, Asian populations exhibit higher allelic frequencies of CYP2C19*2 (21– 45%) and CYP2C19*3 (2– 16%) (Roh et al., 1996; Kimura et al., 1998Roh, 1996 #119)) than European-American populations (*2: 13–19%; *3: 0– 0.3%) (26-Goldstein et al., 1997; Ruas and27- Lechner, 1997; Xie et al., 1999). By contrast, CYP2C9*2 and CYP2C9*3 appear to be more prevalent in European and American populations (8– 15%; 5– 16%) (27-Yasar et al., 1999) than in Asian populations (0; 1– 5%) (28-Sullivan-Klose et al., 1996, Kimura, 1998 #118, Nasu, 1997 #125,{Yoon, 2001 #126). Although the genetic polymorphism of the CYP2C subfamily has been widely studied, there are no data regarding South American populations. Its population is divided ethnically into three main groups: the Amerindian (55 %), Mestizo (30 %) and White (15 %) people. The Amerindian population is represented by approximately 35 ethnic groups, which are clustered based on their linguistic characteristics. The Arawak (mojo), Aymara, Chiquitano, Mataco- Mac'a, Pano, Quechua, Tacana, Tupi-Guarani and Uru- Chipaya are the major ethno- linguistic groups of theAmerindian group. The Mestizo population is the admixture between White and Amerindian populations. This admixture is explained in part by the historical (Aklillu et al., 2003) events of the Spanish Conquest in the Americas that produced a mixture between mostly Quechua and Spaniards. The White population represents a small proportion of the population. In addition to the

arrival of the Spaniards during the Spanish Conquest, a significant number of Europeans immigrated to Boliviabe fore and during World War II.

Mechanism of Action of NSAIDs

Although NSAIDs had been known to inhibit a wide variety of reactions in-vitro, no convincing relationship could be established with their known anti- inflammatory, antipyretic, and analgesic effects until 1971, when Vane and associates and Smith and Willis demonstrated that low concentration of aspirin and indomethacin inhibited the enzymatic production of prostaglandins. There was, at that time, some evidence that prostaglandins participated in the pathogenesis of inflammation and fever, and this reinforced the hypothesis that inhibition of the biosynthesis of these autacoids could explain a number of the clinical action of the drugs (29-Higgs and Vane, 1983). Numerous subsequent observations have reinforced this point of view, including the observation that prostaglandins are released whenever cells are damaged, they appear in inflammatory exudates, and NSAIDs inhibit the biosynthesis of prostaglandins in all cells tested. However, NSAIDs generally do not inhibit the formation of eicosanoids such as the leukotrienes, which also contribute to inflammation, nor do they affect the synthesis of numerous other inflammatory mediation. There are differences of opinion as to whether or not NSAIDs may have other actions that contribute to their therapeutic effects.

EXPERIMENTAL SECTION

Biotransformation was studied in the subcellular fraction termed microsomes, which is a fraction of membrane vesicles corresponding to the endoplasmic reticulum in the intact cell. Microsomes were isolated from rat liver tissues. The impact of genetic polymorphism in one of the enzymes apparently involved in biotransformation of the studied drugs, CYP2C19, was analysed in the used rat liver samples by allelic discrimination. The microsomes were incubated with the substrates Dipyrone, respectively. The produced metabolites were identified and quantified using HPLC analysis. Enzyme kinetic data analysis was finally used to determine enzyme kinetic parameters. More specific details about the material and assays used during this investigations are described within this section.

The samples of rat liver from male Wistar rats weighing between 180 to 220 g, aged 3 months were kindly provided by the Department of Toxicology at the University of Göttingen.

RLM pre-diluted in the same phosphate buffer were added in a volume 25 µl following a previously described method 29-(Evert et al., 1997). The reaction (total volume 100 µl) was initiated by the addition of 25 µl of 10 mM NADPH, dissolved freshly in the same incubation buffer, and allowed to proceed for 10 to 20 min at 37°C in opened Eppendorf tubes in a shaking water bath. In some experiments, the incubation mixture was adjusted to a final volume 200 µl.

The incubations consisted of (final concentration given) 50 mM potassium phosphate buffer (pH 7.4), BE cytochrome P450 (0.4 - 0.6 pmol/ µl), RLM (5 - 10 mg protein /ml), 2 mM NADPH and 25 to 1200 µmol/l 4-methylaminoantipyrine in a final volume of 100 µl.

The reactions were stopped by adding ice-cold acetonitrile (100 µl). The resulting mixture was centrifuged at 13000 rpm for 5 min. 100 µl of the supernatant were used for HPLC analysis. The formation of 4-aminoantipyrine was linear with time between 6 and 10 min.

Incubations with heterologous expressed isolated CYP450s

The recombinant human CYP450 enzymes, pre-diluted in the 50 mM potassium phosphate buffer (pH 7.4), were added in a volume of 25 µl following a previously described method (30-Evert et al., 1997). The reaction (total volume 100 µl) was initiated by the addition of 25 µl of 10 mM NADPH, dissolved freshly in the same incubation buffer, and allowed to proceed for 20 min at 37°C in opened Eppendorf tubes in a shaking water bath

HPLC analysis and chromatographic conditions

HPLC for analysis of Dipyrone metabolites was performed similar as described earlier (31-Asmardi and Jamali, 1983; Geisslinger et al., 1996). The incubation mixtures were centrifuged at 14000 rpm for 5 min. Supernatants were transferred into new tubes and 100 µl used for HPLC analysis. The HPLC system consisted of a L-600A pump (32-Merck, Hitachi Tokyo, Japan) and 655A-40 autosampler (Merck, Hitachi Tokyo, Japan). The system was equipped with a LiChrospher 100 (Å pore size) RP-8e select column with 5 µm particle size (Merck, Darmstadt, Germany)

with internal dimensions of 4 mm x 125 mm preceded by a pre-column (100 Å, diol coated, 5 µM particle size) and some experiments were on a HPLC system consisting of a L-7100 pump (Merck, Darmstadt, Germany) and L-7200 autosampler (Merck, Darmstadt, Germany). The system was equipped with a LiChrospher 100 RP-8e select column 5 µm particle size (Merck, Darmstadt, Germany) preceded by a pre-column (100 Diol, 5 µM). The mobile phase consisted of 75 % (v/v) of 50 mM sodium phosphate buffer (pH 6.0) and 25% (v/v) methanol. The flow rate was 1.0 ml/min. The absorbance was measured at 254 nm with an ultraviolet (UV) detector (655 A Merck Hitachi Tokyo, Japan) linked to computer data system. The injection volume in these analyses was 40 µl, and the retention times of 4-methylaminoantipyrine (4-MAA), 4-aminoantipyrine (4-AA), and internal standard 4-dimethylaminoantipyrine (4-DMAA) were 10.30, 7.70 and 16.75 minutes, respectively.

RESULTS

Investigations of the metabolism of Dipyrone **by RLM**

HPLC Analysis. The substances 4-aminoantipyrine (4-AA) (peak 1, Fig. 2) and 4-methylaminoantipyrine (4-MAA) (peak 2) have been measured with HPLC as illustrated in a chromatogram obtained from injection of 20 µl of standard solution with 25 µmol/l of 4-AA and 4-MAA, each. As shown in the figure, the separation was completed within 10 min. The retention times were 5.08 min for 4-AA and 6.72 min for 4-MAA.

Investigations of the metabolism of by recombinant Human CYP450s

To confirm the specific CYP enzyme involved in biotransformation of 4-methyl-aminoantipyrine, which appeared to be the enzyme CYP2C19 according to the inhibition study and according to one experiment with human liver microsomes from a CYP2C19 deficient subject, microsomes expressing individualrecombinant human P450 isozymes (CYP1A1, CYP1B1, CYP1A2, CYP2A6,CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A5 and CYP3A7) were incubated with different concentrations of 4-MAA from 25 to 800 µmol/l in the presence of an NADPH-regenerating system at 37°Cfor 20 min. Some formation of 4-AA was observed in theincubations with (CYP1A1, CYP1B1, CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1,CYP3A4, CYP3A5 and CYP3A7) whereas the high formation of 4-AA was observedonly with CYP2C19 and with CYP1A2, data are given in Fig.4 .

The formationrates of 4-aminoantipyrine (AA) with rCYP2C19 were faster thanwith the other P450 isozymes. Also, the highest catalytic efficiency(intrinsic clearance, V_{max}/K_m) was observed with CYP2C19, (0.077 µl/min/pmol).

The average immunoquantified levels of the various specific P450s in human liver microsomal samples were 25, 42, 1.2, 1.43, 6.7, 16.85, 17.88, 31.29, 2, 26.82, 33.63, and 96 pmol/mg proteins in human liver for the CYP2C19, CYP1A2, CYP1B1, CYP1A1, CYP2D6, CYP2C8, the order of cytochrome P450 enzymes as above. In conclusion of our in vitro investigations, cytochrome P450 2C19 appeared as the primary enzyme metabolizing metamizole. 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 4-methylaminoantipyrine (50 µM) was incubated with rat liver microsomes (5 mg/ml of microsomal protein) at 37 °C for 20 min and the metabolites were analysed by HPLC after extraction. No metabolites were seen when 4-methylaminoantipyrine and microsomes were incubated without NADPH and with NADPH but without any incubation time (0 min). In rat liver microsomes, the metabolism of 4-methyaminoantipyrine was strongly inhibited by a concentration of 50 µM omeprazole as shown in Fig. 10, the inhibition was (65 % inhibition) and to a lesser degree by ketoconazole (37 % inhibition) and but no inhibition was detected with alpha-naphthoflavone, coumarin, quinidine and sulphaphenazoleas shown in Table.2

Figures and Tables

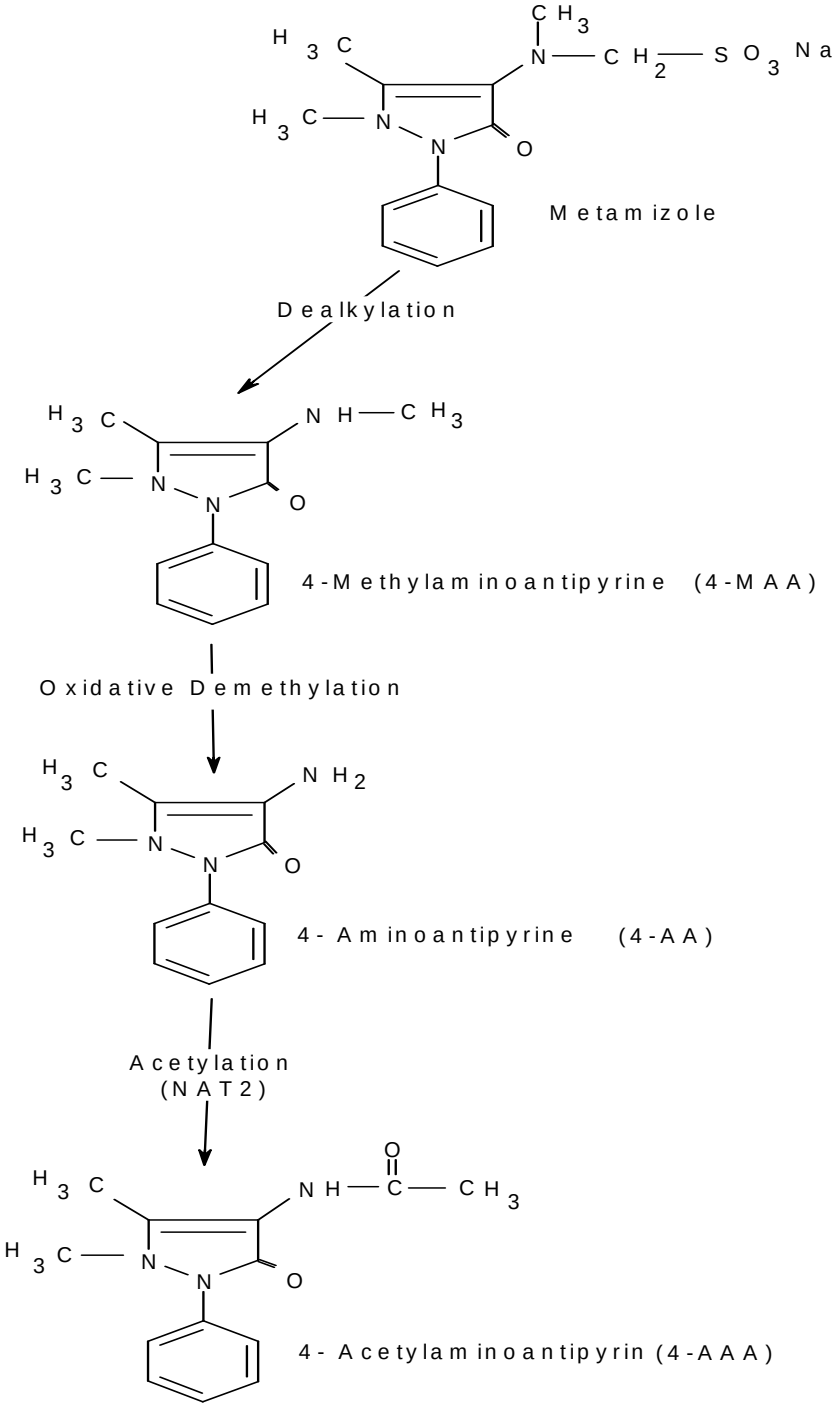

Fig. 1 Structure and biotransformation of Dipyrone and its main metabolites in man, drawn according to (Geisslinger et al., 1996)

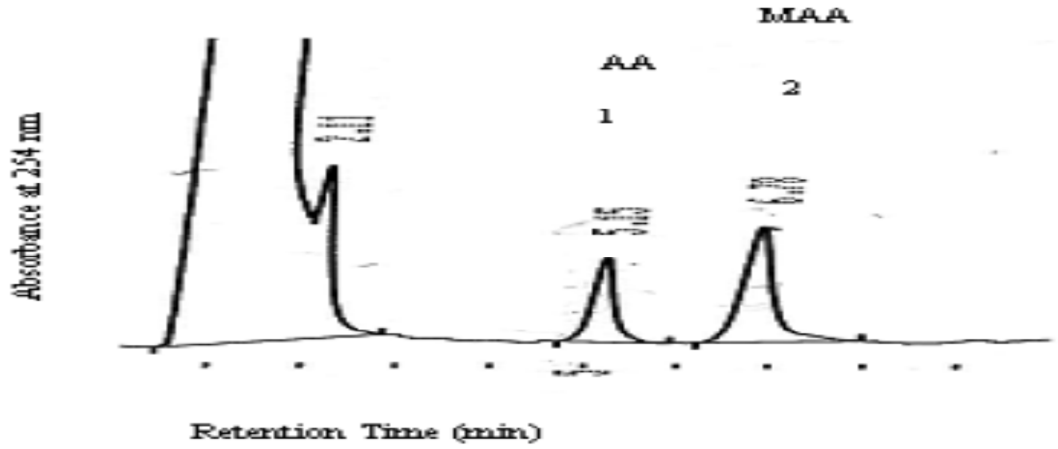

Fig. 2 Elution profiles of 4-methylaminoantipyrine and its metabolites by HPLC with a RP-8 endcapped (5 µm particle size, 125 x 4 mm internal dimensions) column equipped with a pre-column (100 Å pore size, diol-coated, 5 µm particle size). The mobile phase consisted of 80 % (v/v) of 25 mM sodium phosphate buffer (pH 6.0), 15% acetonitrile and 5% (v/v) methanol. The flow rate was 1.0 ml/min. The absorbance was measured at 254 nm with a detector linked to a computer data system. The chromatogram shows 4-methylaminoantipyrine and its metabolite formed by rat liver microsomes. A reaction mixture (200 µl) with 1.25 mg/ml (final concentration) of microsomal protein from male Wistar rats, 1.0 mg/ml of NADPH and 50 µmol of 4-methylaminoantipyrine was incubated for 20 min at 37 °C in 25 mM potassium phosphate buffer (pH 7.4). 4-methylaminoantipyrine metabolites were extracted and analyzed by HPLC. Peaks: 4-AA at 5.15 min and 4-MAA at 6.78 min

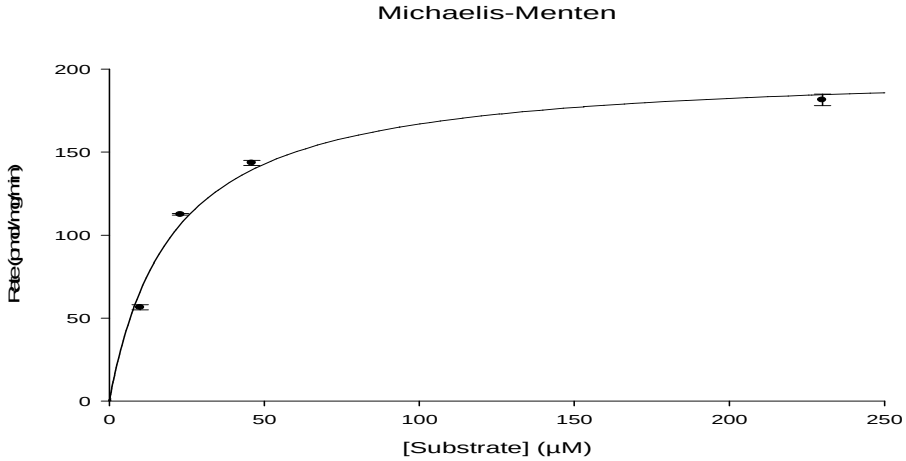

Fig. 3 Kinetic plot of demethylation of 4-methylaminoantipyrine by RLM. The reaction mixture (200 µl) included 1.25 mg/ml (final concentration) of microsomal protein of male Wistar rats and 1.0 mg/ml of NADPH. 4-methylaminoantipyrine was incubated for 20 min at 37 °C in 25 mM potassium phosphate buffer (pH 7.4). 4-methylaminoantipyrine metabolites were extracted and analyzed by HPLC.

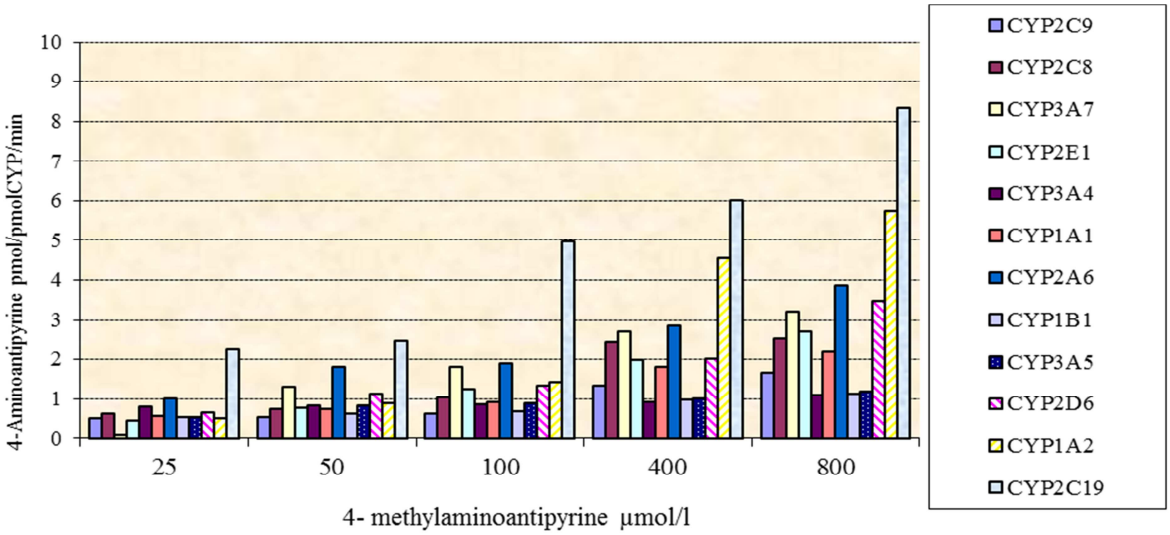

Fig. 4 Cytochrome P450 isozymes in the in-vitro demethylation of methylaminoantipyrine. MAA was incubated with microsomes expressing human recombinant P450 isozymes (0.6 pmol/µl) for 20 min and the concentration of 4-methylaminoantipyrine was 25, 50, 100, 400, 800 µmol/l. The formation of 4-aminantipyrine AA was monitored by HPLC analysis with UV detection. Results are as average of duplicate incubation

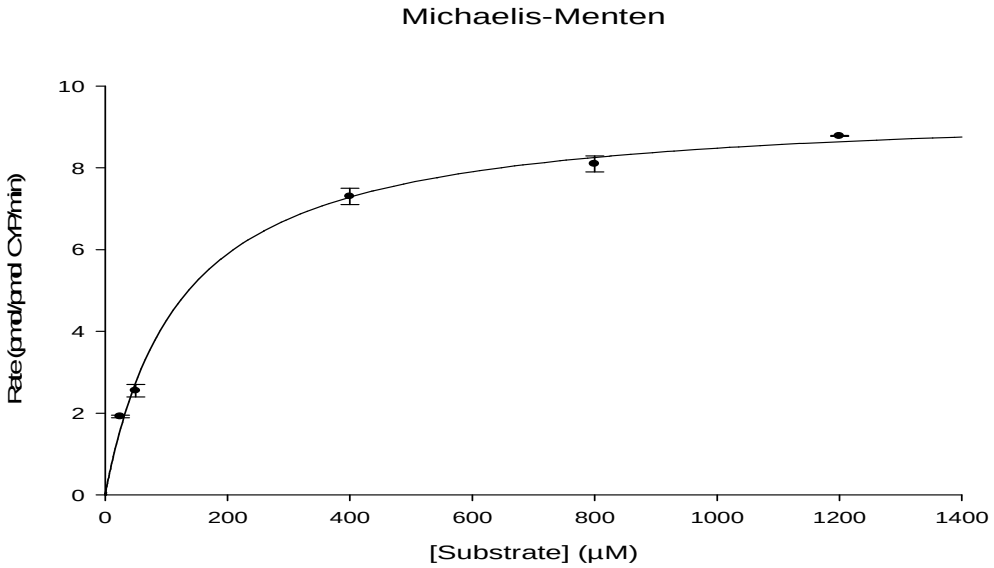

Fig. 5 Kinetic plot of demethylation of 4-methylaminoantipyrine by recombinant Human CYP2C19 enzyme. MAA was incubated with human recombinant CYP2C19 enzymes (0.6 pmol/µl) for 20 min and the concentration of 4-methyaminoantipyrine was 25, 50, 100, 400, 800, 1200 µmol/l

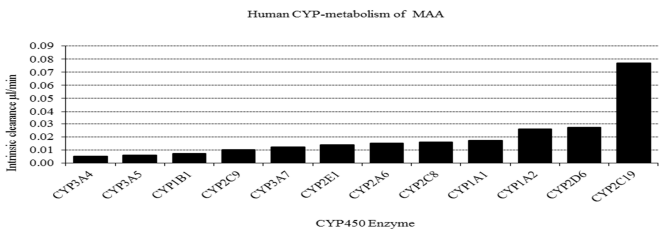

Fig.. 6 Intrinsic clearance of 4-methylaminoantipyrine by specific cytochrome P450 enzyme after incubation. 4-MAA with microsomes expressing human recombinant P450 isozymes (0.6 pmol/µl) for 20 min and the formation of aminantipyrine (AA) was monitored by HPLC analysis with UV detection. Results are given as average of duplicate incubation

Table 1 Enzyme kinetic parameters for Dipyrone demethylation by cytochrome P450. The dipyrone concentration range varied from 25 to 800 µM. The concentration of baculovirus-expressed enzymes was 0.6 pmol/µl. All data represent the mean of minimally two experiments

Enzyme	V_{max} (pmol/pmolCYP/min)	K_m (µmol/l)	Cl_{int} (µl/pmol CYP/min)	Cl extrapolated (l/min)
CYP2C19	9.5	123	0.077	4.634
CYP2D6	3.7	138	0.027	0.269
CYP1A2	8.1	317	0.026	1.199
CYP1A1	2.6	150	0.017	0.465
CYP2C8	4	245	0.016	0.413
CYP2A6	3.8	260	0.015	0.588
CYP2E1	2.3	169	0.014	0.510
CYP3A7	2.2	177	0.012	1.790
CYP2C9	2.2	216	0.010	0.031
CYP1B1	1.4	193	0.007	0.614
CYP3A5	1.5	263	0.006	0.012
CYP3A4	1.6	315	0.005	0.009

Table 2 Estimated % inhibition of the formation of 4-aminoantipyrine by selective chemical inhibitors added at a concentration of 50 µM. A reaction mixture (200 µl), 5 mg/ml of microsomal protein of male Wistar rats, and 50 µmol of 4-methylaminoantipyrine was incubated for 20 min at 37°C in 25 mM potassium phosphate buffer, (pH 7.4) 4-methylaminoantipyrine metabolites were extracted and analyzed by HPL

RLM			
Inhibitors	**% Inhibition**	**K_i (mM)**	**IC_{50} (mM)**
Omeprazole	65.90	0.04	0.05
Ketoconazole	36.60	0.14	0.77
Sulphaphenazole	(no inhibition)	-	-
Coumarin	(no inhibition)	-	-
Quinidine	(no inhibition)	-	-
Alpha-naphthoflavone	(no inhibition)	-	-

DISCUSSION

Dipyrone is used for human therapy in several European countries. In addition to the use of these drugs for postoperative pain, they are currently used in self medication (Rodzynek et al., 1986; Lane, 1988)and functional hepatocellular mass (Krahenbuhl et al., 1989) in several diseases such as liver cirrhosis (Urbain et al., 1990), chronic hepatitis (Lashner et al., 1988), and hepatocarcinoma (Feuer, 1988; Agundez et al., 1994). In agreement with our invitro data large, interindividual differences in the capacity to detoxify such drugs have been reported (Goldberg and Brown, 1987; Lashner et al., 1988; Cotting et al., 1990; Merkel et al., 1992). Considering that the main urinary metabolites in man are acetylated and formylated as shown by others (Volz and Kellner, 1980; Cotting et al., 1990; Merkel et al., 1992; Zylber-Katz et al., 1992; Agundez et al., 1994; Agundez et al., 1995; Costa et al., 2006) who studied in-vivo the plasma concentrations of pyrazolones such as aminopyrine and metamizole or metabolism in liver disease. We have studied in vitro the oxidative biotransformation of the metamizole and aminopyrine by genetically polymorphic enzymes.

Investigations of the metabolism by RLM

The rat liver microsomes (RLM) is used widely to characterize the role of cytochrome P450s (P450) and other enzymes in drug metabolism. The resulting supernatant is then centrifuged at a higher force to precipitate the microsomes. The microsomal pellet is resuspended in a final suspension buffer and is then ready for use. The complex metabolism of dipyrone has been the subject of many in-vivo studies. However, the specific cytochrome P450 enzymes involved catalysing the formation of 4-AA from 4-MAA is still not unequivocally identified.

The establishment of appropriate HPLC analytical methods started with the quantification of the reference substances of 4-aminoantipyrine and 4- methylaminoantipyrine. The separation was completed with in 13 min. The relative order of retention times was AA at 5.08 min and MAA at 6.72 min, as shown in Fig. 6 in results. Similar results were observed in the analysis of dipironee metabolites to study their formation in human liver microsomes (Geisslinger et al., 1996). For the incubations of diprone with rat liver microsomes we used two different preparations of rat liver microsomes. Mean V_{max} (arithmetic mean of 5 incubations) was 201(standard deviation, SD, 42.2 pmol/mg protein/min) and mean K_M was 20.9 (SD 3.8) µmol/l are shown in Fig. 3. The corresponding intrinsic

clearance was 9.61 µl/mg protein/min (SD 3.9). The quantity of microsomes protein was 1.25 mg protein. Analysis of metamizole metabolism was performed with hepatic microsomes of untreated male Wistar rats weighing between 180 to 220 g, aged 3 months, because the female rats had lower N-demethylation activity of 4-methylaminoantipyrine than male rats (Imaoka et al., 1988). The two control incubation samples without NADPH and zero incubation time are shown in Fig. 9 at the right and left part, respectively. 4-methylaminopyrine (50 µmol/l) was incubated with rat liver microsomes 5 mg/ml of microsomal protein at 37°C for 20 min and the metabolites were analyzed by HPLC after extraction. The metabolite was not seen when 4-methylaminoantipyrine and microsomes were incubated without NADPH and with NADPH but incubation time zero. This metabolite was almost not seen in an assay system with NADPH but its formation inhibited by omeprazole, Omeprazole is a strong inhibitor of CYP2C19 (Imaoka et al., 1988). The formation of 4-aminoantipyrine from 4-methylaminoantipyrine was strongly inhibited by a concentration of 50 µM omeprazole (65 % inhibition) and to a lesser degree by ketoconazole (37% inhibition) but no inhibition was detected with alpha-naphthoflavone, coumarin, quinidine and sulphaphenazole (Table 2). However, our results indicated that CYP2C19 represents the main contributor to the of metabolism of Dipyrone in rat liver

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