International Scholars Journals

African Journal of Biology ISSN 2167-0413 Vol. 2 (4), pp. 148-156, April, 2015. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Kinetic parameters of human P450 isoforms involved in the metabolism of the antiallergic drug, loratadine

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Accepted 21 March, 2015

The kinetics of loratadine to de sloratadine was studied with human liver microsomes using substrate concentrations in the range of 0-50 µM. The specific cytochrome P450 (CYP) isoforms mediating the biotransformations were identified using microsome s containing specific recombinant CYP isozymes expressed in human lymphobla stoid cells, and by the use of CYP isoform -selective chemical inhibitors. In this study, the kinetic analysis of loratadine metabolism was performed by measuring the disappearance rate of parent compound (loratadine) and the production rate of its major metabolite (desloratadine), and it was found that the Michaelis-menten Km and Vmax values were 18.20 µM and 2169 pmol/min/mg for loratadine disappearance, and 25.20 µM and 486.98 pmol/min/mg for desloratadine formation, respectively. Among the recombinant CYPs, CYP3A4 and CYP2D6 exhibited the highest metabolic activity of loratadine, though no detectable activity was observed for CYPs 2C8, 2C9 and 2E1. Further kinetic analysis revealed first, that the clearance (Vmax/Km) values for CYP3A4 were 135.7 µl/min/mg protein for loratadine disappearance and 12.25 µl/min/mg protein for desloratadine formation, and secondly, that the clearance (Vmax/Km) values for CYP2D6 were 15.45 µl/min/mg protein for loratadine disappearance and 5 µl/min/mg protein for desloratadine formation. In addition, the formation of de sloratadine was inhibited by 2 µM ketoconazole (a CYP3A4 inhibitor) and 10 µM quinidine (a CYP2D6 inhibitor) by 66.43 and 33.03%, re spectively. Facts obtained in this study showed that this is the first time that kinetic parameters are described. In effect, this approach based on the disappearance rate of parent compound and production rate of major metabolite appears to be useful for detailing the kinetics of older drugs, for which detailed metabolic profiles have not been reported.

Key words: Desloratadine, kinetics, loratadine, isoform.

INTRODUCTION

Loratadine is a onc e-a-day nonsedating antihistamine, indicated for the relief of nasal and non-nasal symptoms of s easonal allergic rhinitis, with no autonomic anticholinergic effects (Berdard et al., 1985; Batenhorst et al., 1986; Villani et al., 1986; Clissold et al., 1989; Haria et al., 1994; Kay and Harris, 1999). Studies on the metabolism (Katchen et al., 1985; Hilbert et al., 1987; Weyer et al., 1992; Simons and Simons, 1999) have shown that loratadine is rapidly absorbed and undergoes extensive first-pass metabolism to Descarboethoxyloratadine (desloratadine). Desloratadine is also pharmacologically active and is present in the plasma at low concentrations due to metabolism to several hydroxylated metabolites (Katchen et al., 1985; Ramanathan et al., 2005). Human cytochrome P450 3A4 and 2D6 was demonstrated to be the major isoform involved in desloratadine formation with a Km range for loratadine of 7 to 35 µM (Yumibe et al., 1995, 1996; Barecki et al., 2001; Ghosal et al., 2003). Loratadine is 97 to 99% plasma protein bound with an apparent oral clearance of 142.0 ± 56. 5 µl/min/kg for a 40-mg dose. The clinical plasma Cmax for a 10 mg dose for loratadine and desloratadine are 4.7 and 4 ng/ml, respectively

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(Hilbert et al., 1987). The identification of the CYP enzymes involved in drug metabolism is an important step for better understanding and prediction of the potential metabolic-based drug-drug interactions and for investigating the potential impact of genetic polymorphisms (Ingelman -Sundberg, 2002; Evans et al., 2003; Bjornsson et al., 2003).Advances in mass spectrometry c ontinue to bring new c apabilities to drug study. Due to its excellent sensitivity and selectivity, liquid chromatography-mass spectrometry (LC-MS/MS) is undoubtedly a convenient technique for compound detection and identification. In the first part of this study (companion paper), it was shown that a hy brid linear ion-trap-triple quadrupole mass spectrometer coupled to HPLC allowed identification of phase I metabolites after single injection of an extract of human liver microsomes (HLM) incubation medium (qualitative analysis).

As a first step to the investigation of the factors involved in loratadine side effects, such as genetic polymorphism and drug interactions, the aims of the second part of this study are to: (1) characterize all the hepatic CYP isoforms involved in loratadine metabolism, (2) systematically investigate the enzyme kinetics of the main metabolic pathways and to compare their implication in both desloratadine production and loratadine consumption, and (3) illustrate the potential of turbulent flow chromatography-tandem mass spectrometry (TFC-MS/MS) for metabolism kinetic studies.

The aim of this study is, therefore, to identify all the hepatic isoforms involved in loratadine metabolism, as well as to systematically investigate the enzymology of the metabolic pathways. These data form a better basis to assess risk factors of loratadine abuse, such as genetic polymorphism and drug interactions.

MATERIALS AND METHODS

Chemicals and microsomes

NADPH, methylclonazepam, ketoconazole and quinidine were purchased from Sigma Chemical Co. (St. Louis, MO). Loratadine [4-(8-chloro-5,6-dihydro-11h-benzo[5, 6]cyclohepta[1,2-b]pyridin-11-ylidene)-1piperidinecaboxylic acid ethyl ester, SCH 29851] and desloratadine (descarboethoxyloratadine, SCH 34117) were obtained from Ibn El Baytar (Tunisie). All other reagents were of the highest purity commercially available.

Pooled HLM as well as microsomes prepared from baculovirus-infected ins ect cells (Supersomes®) expressing the human CYP1A2, 2B6, 2C8, 2C9*1, 2C19, 2D6*1, 2E1, 3A4 or 3A5, and control microsomes from wild-type insect cells were purchased from BD Gentest (Woburn, MA, U.S.A.). All CYPs were co-expressed with NADPH-cytochrome *c* reductase. CYP 2E1, 2C8, 2C9 and 3A4 were also co-expressed with cytochrome b5.

Incubation procedure s with microsome s or recombinant CYPs for characterizing metabolic pathways

The standard incubation mixture (250 µl) consisted of 0.1 M Hepes buffer pH 7.4 at 37°C, 10 mM $MgCl_2$, loratadine (0-40 µM) and microsomes (HLM or Supersomes®). Substrate and microsomes were preincubated at 37°C for 5 min before starting the reaction by adding NADPH (1 mM). Incubations were stopped after 30 min at 37°C by adding 125 µL of acetonitrile, vortexed, centrifuged at 3500 rpm, and immediately frozen.

Control inc ubations without NADPH or HLM were performed to test the chemic al stability of loratadine in the medium. All the incubations were conducted in duplicates.

To investigate the involvement of particular CYP isoforms in loratadine metabolism, 5 µM loratadine was incubated with the different recombinant CYPs at a concentration of 50 pmol/ml (as recommended by the supplier) for 30 min in a total volume of 250 µl.

Analysis of loratadine metabolism kinetics

In preliminary experiments, the linearity of metabolism kinetics was checked at 5 µM loratadine using a range of CYP contents (10-50 pmol/ml), micros omal protein concentrations (0.1-1 mg/ml) and incubation time (0-60 min). The velocity (V) of both loratadine disappearance and desloratadine formation were then determined at loratadine concentrations ranging from 0 to 40 µM using 0.25 mg/ml microsomes, 40 pmol/ml CYP 3A4 or 15 pmol/ml CYP 2D6. The incubation time was 15 min for CYP 2D6 and 30 min for both microsomes and CYP 3A4.

The results obtained with recombinant CYP were scaled to HLM using the previously described relative activity factor (RAF) approach (Crespi, 1995; Nakajima et al., 2002). RAF values for all CYP isoforms were obtained from BD Gentest. The individual CYP scaled reaction (V) was calculated by multiplying the experimental reaction (V) by the RAF_i value. This scaling process integrates the hepatic abundance of each CYP isoform and the differences in activity between cDNA -expressed enzymes and human liver microsomal CYP. Results were model -fitted to the Michaelis-Menten equation and the kinetic parameters calculated by nonlinear regression analysis using Winreg 3. 1 (available online: http://www.unilim.fr/pages_pers o/jean. debord/winreg/winreg1.htm,). The intrinsic clearance (Cl_{int}) was estimated as the V_{max}/K_m ratio.

Following the RAF approach, the relative contributions (RC_i) of CYP 3A4 and CYP 2D6 to both loratadine consumption and desloratadine production were estimated using the following equation:

$$RC_i = \frac{Cl_{int}\ CYP_i}{Cl_{int}\ HLM} \times 100$$

Table 1. Contr ibution of CY P isofor ms to the for mation of different loratadine metabolites (+, involved; -, not involved).

Cytochromes P450 / Metabolites	1A2	2B6	2C8	2C9	2C19	2D6	2E1	3A4	3A5
M4/M5/M6/M9/M10: Hydroxy-desloratadine	-	-	-	-	+	+	-	+	-
M1: Dihydroxy-desloratadine	+	+	-	-	-	+	-	+	+
M2/M3/M14: Dihydroxy-loratadine	-	-	-	-	-	+	-	+	-
M7: Desloratadine	+	+	-	-	+	+	-	+	+
M8/M11/M12/M17/M18/M19: Hydroxy-loratadine	+	+	-	-	+	+	-	+	+
M16: Piperidine-oxide of piperidine aromatized desloratadine	-	-	-	-	-	+	-	+	+
M13: Aliphatic hydroxide of M15	-	+	-	-	-	+	-	+	+
M15: Loratadine-carboxylic acid	-	-	-	-	-	-	-	+	+

Effects of chemical inhibitors

This study investigated the effect of quinidine and ketoconazole, as typical inhibitors of CYP 2D6 and CYP 3A4, respectively (Newton et al., 1995; Schmider et al., 1995), on loratadine (5 µM) metabolism by HLM. Ketoconazole (0.25 - 2 µM) and quinidine (1 - 10 µM) were dissolved in DMSO and added to the incubation media so that the final content of DMSO was less than 0.1%. Control incubations were spiked with the same amount of DMSO. Loratadine and desloratadine concentrations were measured in the incubation medium after the reaction had been stopped, and the perc entage inhibition of loratadine metabolism, as well as desloratadine production, was calculated with respect to control.

Analytical methods

Loratadine and desloratadine determination was performed using TFC-MS/MS. The system used consisted of a Cohesive 2300 system (Cohesive technologies, Milton Keynes, U.K.) comprising a CTC HTC PAL autosampler kept at 6°C, 2 binary high-pressure Agilent 1100 pumps, and 3 six -port switching valves controlled by the Aria OS software package. System configuration, parameters and analytical process were previously described in details (Sauvage et al., 2006). Briefly, online extraction was performed at a high flow-rate (1.25 ml/min) on a Cyclone®, 50 -µm particle size (50 × 0.5 mm I. D.) column (Cohesive technologies) in alkaline conditions (phase A1: 20 mM ammonium acetate in water with 0.1% ammonia; phase B1: mixture of acetone/acetonitrile/propanol -2 [50:30:20; vol/vol/vol]). Chromatographic separation was performed in acidic conditions (phas e A2: 0.1% formic acid in water; phase B2: 0.1% formic acid in acetonitrile) using an Xterra MS C18, 5 µm (50 × 2.1 mm I.D.) column (Waters, Milford, MA) kept at 45°C, with a constant flow rate of 400 µl/min.

Detection was performed with a TSQ Quantum Discovery MS/MS system (Thermo-Electron, Les Ulis, Franc e) equipped with an orthogonal electrospray ionization source and controlled by the Xcalibur computer program. MS/MS detection was performed in the positive ion, multiple reaction monitoring (MRM) mode following two transitions for loratadine (m/z 382>337 m/z; 382>267), desloratadine (m/z 311>259 m/z; 311>243) and the internal standard (IS) methylclonaz epan (m/z 329>279 m/z; 329>221).

Owing to the first step of turbulent-flow chromatography which involves sample purification and concentration on a re-usable extraction column, incubation supernatant could be injected after protein precipitation by addition of equal volumes of acetonitrile and methylclonaz epam (100 ng/ml in acetonitrile). Calibration standards were prepared at 0, 1, 2.5, 5, 10, 25, 50, 100, 250 and 500 ng/ml. The limit of quantitation was 1 ng/ml and calibration curves obtained using quadratic regression gave squared correlation coefficients > 0. 999 over the 1 to 500 ng/mL range. The method showed good inter-assay precision and accuracy with relative standard deviation values ranging from 0.9 to 9.5% and mean relative errors from -2 to 2.5% over the linearity range. Intra-assay precision and accuracy were assessed by analysing incubation medium spiked at 3 concentration levels (1, 100 and 500 ng/ml) on the same day and were also satisfactory with relative standard deviation values ranging from 4 to 12% and mean relative errors always < 15%.

RESULTS

Loratadine metabolism

Loratadine was stable in control incubations without NADPH or HLM, showing no decrease in the concentration of the unc hanged compound and no production of the metabolite.

Nineteen metabolites were generated by the NADPH-dependent enzyme reaction (see companion paper). Desloratadine was the major metabolite of loratadine (m/z 311, eluted at 4.75 min).

As shown in Table 1, CYP 1A2, 2B6, 2D6*1, 2C19, 3A5 and 3A4 were involved in loratadine metabolism and

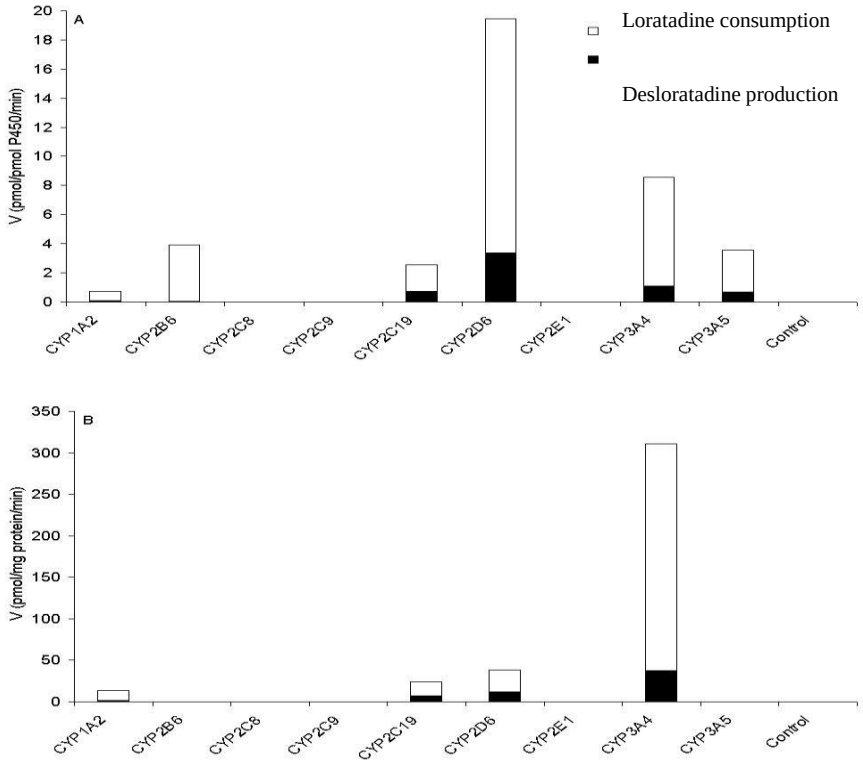

Figure 1. Comparison betw een the amount of desloratadine produced and the amount of loratadine consumed over 30 min incubation of loratadine w ith different CYP, before (A) and follow ing (B) the RAF method.

desloratadine formation, while CYP 2C8, 2C9 and 2E1 were not. Interestingly, CYP 3A4 was involved in the formation of all the metabolites (Table 1). The relative metabolic activities of human CYP for loratadine consumption as well as desloratadine p roduction are shown in Figure 1A and B.

Before scaling to HLM by the RAF values, CYP 2D6 exhibited the highest catalytic activity for l oratadine disappearanc e and desloratadine formation with 18.5 and 3 pmol/pmol P450/mn, respectively, followed by CYP 3A4 with 8 and 1 pmol/pmol P450/min for loratadine disappearanc e and desloratadine formation, respectively (Figure 1A).

The (V) of individual CYP isoforms involved in loratadine metabolism scaled to HLM by the RAF values is shown in Figure 1B. CYP 3A4 ex hibited the highest catalytic activity for loratadine disappearance (273. 46 pmol/mg protein/min), followed by 2D6 with 26. 90 pmol/mg protein/min. Consistently, the formation of desloratadine was catalyzed most efficiently by CYP3A4 (37 pmol/mg protein/min) and CYP2D6 (11.09 pmol/mg protein/min), followed by CYP2C19 (6.49 pmol/mg protein/min) and CYP1A2 (0.67 pmol/mg protein/min).

Enzyme kinetics

HLM displayed K_m, V_{max} and Cl_{int} for loratadine disappearanc e of 18 µM, 2169 pmol/mg protein/min and 119 µl/min/mg protein, res pectively. Desloratadine production displayed a similar K_m (25 µM), but a 4.4-fold lower reaction velocity (487 pmol/mg protein/min) and a 6.15-fold lower Cl_{int} (19.32 µl/min/mg protein) (Figure 2A, Table 2).

Relative contribution of CYP 3A4 and 2D6 to loratadine metabolism

The (RC_i) of microsomal CYP 3A4 and CYP 2D6 to desloratadine production as well as loratadine depletion was calculated by means of the enzyme kinetic parameters estimated using recombinant human CYP (Table 2). The Cl_{int} of loratadine disappearance by CYP 2D6 was approx. 3-fold higher than that of desloratadine formation (15.4 versus 5.46 µl/min/mg protein) (Figure 2B, Table 2). For CYP 3A4, the Cl_{int} of loratadine disappearanc e was approximately 11-fold higher than that of desloratadine formation (135.7 versus 12.2 µl/min/mg protein) (Figure 2C, Table 2).

The RCi for desloratadine production and loratadine disappearanc e by CYP 2D6 and CYP 3A4 were 13 and 26%, and 63 and 114%, respectively.

Chemical inhibition of loratadine metabolism by HLM

The respective contribution of CYP 3A4 and CYP 2D6 to

Figure 1

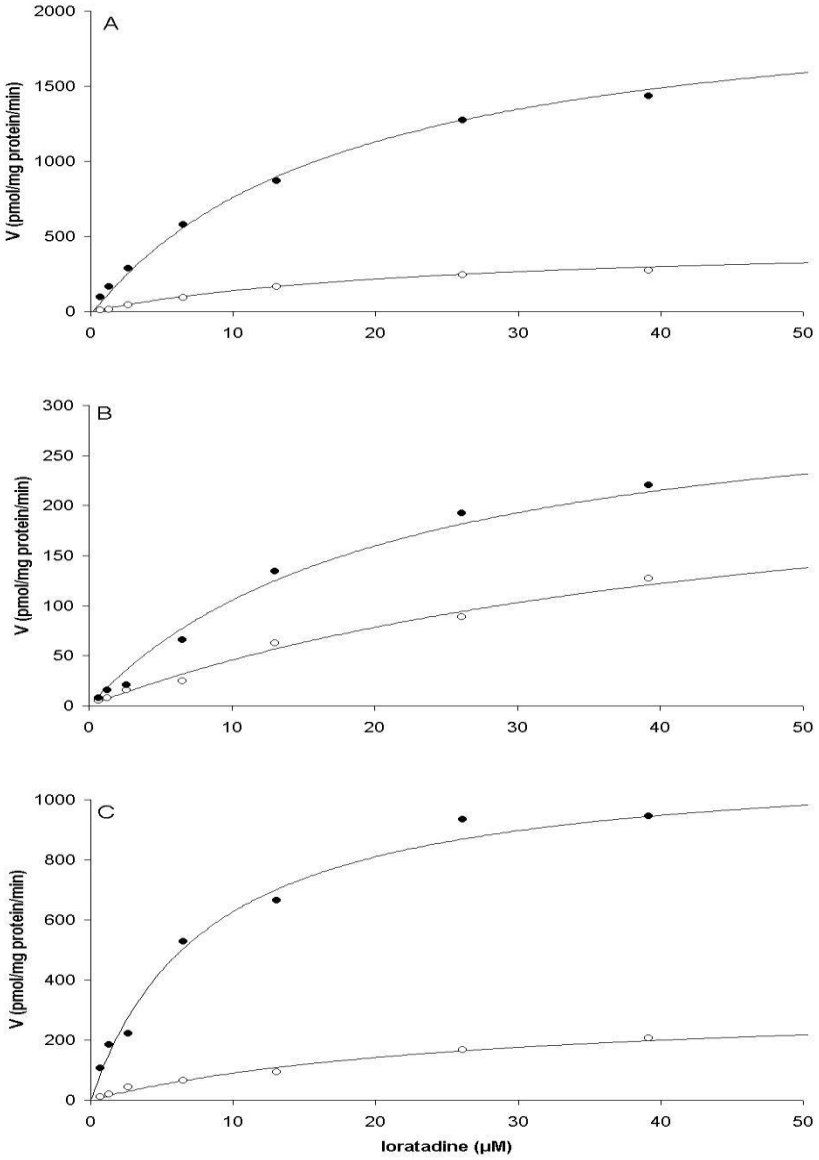

Figure 2. Enzy matic kinetic modelling of loratadine disappearance (solid circles), and desloratadine for mation (empty circles) w ith human liver microsomes (A), CY P 2D6 (B) and CY P 3A4 (C) Supersomes using the Michaelis -Menten function. (A and B) results are presented as the mean of duplicates obtained w ith recombinant CY P and scaled to HLM follow ing the RA F method.

Figure 2

Table 2. Kinetic parameters (mean estimate ± standar d error) computed from loratadine incubation w ith human liver microsomes, CY P 3A4 and 2D6 Sup ersomes and scaled to HLM using the RA F.

Parameter	Effect	Km (µM)	Vm pmol/min/mg protein	Cl_{int} µl/min/mg protein
HLM	Loratadine disappearance	18 ± 1.0	2169 ± 59.5	119
	Desloratadine formation	25 ± 2.3	487 ± 31.3	19
CYP2D6	Loratadine disappearance	21.3 ± 4.5	330.1 ± 29.9	15.4
	Desloratadine formation	50.6 ± 11.8	277 ± 38.0	5
CYP3A4	Loratadine disappearance	8.2 ± 1.2	1114 ± 51.9	135.7
	Desloratadine formation	27.4 ± 5.7	336.4 ± 32.9	12.2

Table 3. Inhibition of loratadine metabolis m by ketoconazole and quinidine. Results are expressed as percentage inhibition of desloratadine production by pooled human liver microsomes *in vitro* (mean of duplicates).

Parameter		
Ketoconazole (µM)	**% inhibition of desloratadine production**	**% inhibition of loratadine disappearance**
0.25	9.9 (8.7, 11.04)	21.2 (19.5, 22.9)
0.5	37.7 (35, 40.4)	59.4 (54.6, 64.2)
1	41.6 (40.1, 43.1)	69.9 (68.5, 71.3)
1.5	54.5 (51.6, 57.4)	78.5 (72.9, 84.1)
2	66.4 (62.3, 70.5)	84.3 (78.4, 90.2)
Quinidine (µM)		
1	5.4 (3.6, 7.2)	9.8 (5.4, 14.2)
5	24.1 (18.7, 29.5)	13.5 (10.4, 16.6)
10	33.0 (29.8, 36.2)	16.5 (14.5, 18.5)

desloratadine formation as well as to loratadine metabolism was also evaluated using selective chemical inhibitors (Table 3).

The inhibitory effect of ketoc onazole at each concentration tested on loratadine consumption (with respect to the control experiment without mic rosomes, where no loratadine was metabolised) was greater than that of its inhibitory effect on desloratadine production (Table 3).

Ketoconazole at 2 µM inhibited the loratadine disappearanc e and the desloratadine production by 66.4 and 84. 3%, respectively (Table 3). At the highest concentration tested (10 µM), quinidine inhibition of desloratadine formation reached 33%, while the maximal inhibition of loratadine consumption was only 16.5% (Table 3).

DISCUSSION

In this study, a sensitive, specific and totally automated liquid chromatography - mass spectrometry (LC-MS/MS) technique was developed for the determination of loratadine and desloratadine, which allowed for fast analysis of a very large number of small-volume incubation samples with low drug concentrations, without extraction. It proved to be very powerful for loratadine metabolism pathways characterization and kinetic analysis, the latter being estimated by measuring both the disappearance rate of loratadine and the production rate of its major metabolite.

This study provides, for the first time, a detailed appraisal of loratadine metabolic pathways in humans, showing the contribution of CYP 1A2, 2B6, 2C19, 2D6, 3A4 and 3A5, most of which are polymorphic or are subject to drug induction or inhibition, which might be responsible for inter-individual differences in drug response and drug-drug interactions.

Desloratadine production by HLM represents 16% of the amount of loratadine consumed (19 versus 119 µl/min/mg protein). This result proves that HLM metabolis e loratadine more efficiently than they produce desloratadine, which is only one of the phase I metabolites of loratadine (see the first part of this study in companion paper).

The approach based on the disappearance rate of the parent drug from the incubation medium presented some advantages here, considering that: some metabolites formed are not available as pure compound to set up a determination method, and loratadine metabolic pathways are many and result in a large number of metabolites. To determine the kinetic parameters, a commercial pool of HLM prepared from 29 donors, which is more representative than HLM from a single or a few donors was used.

It is important to extrapolate data obtained with recombinant CYP450 to HLM. One approach is bas ed on the abundanc e of the CYP proteins in HLM (Shimada et al., 1994; Rendic, 2002), and the other is the relative activity factor (RAF), propos ed by Cres pi (Cres pi, 1995). The prediction using RAF was found to be superior to the former (Nakajima, 2002). In this study, different approaches mainly used for reaction phenotyping were applied: RAF, relative contribution of each enzyme (RCi) and diagnostic inhibitors. Each of these approaches has some limitations (Xue-Qing Li et al., 2003), which is why they were used in combination.

CYP 3A4 and 2D6 appear to be the prominent CYP isoforms involved in the metabolic pathway of loratadine in HLM. This study found a high activity of CYP 3A5 and CYP 2B6 in loratadine metabolism, but the RAF approach could not provide a quantitative estimate of their involvement in loratadine metabolism. CYP 3A5 is generally a minor component of total CYP 3A (Wrighton et al., 1990), only expressed in approximately 20% of individuals of European origin and with an expression level usually lower than that of CYP 3A4 (Gorski et al., 1994), except maybe in the intestinal wall.

Kinetic analysis showed that CYP 3A4/2D6

metabolis ed loratadine more efficiently than they produced desloratadine (for CYP 3A4: Clint = 12.2 versus 135.7 µl/min/mg protein; for CYP2D6: Clint = 5 versus 15.4 µl/min/mg protein). These results are in agreement with the fact that both CYP 3A4 and CYP 2D6 are implicated in the formation of the other metabolites of loratadine. The sum (CYP 2D6 and 3A4) of Cl_{int} values for desloratadine production is 89% that of desloratadine production by HLM, showing that CYP 3A4 and CYP 2D6 are the principle CYP involved in desloratadine production, and confirming the smaller involvement of other metabolic pathways in this specific reaction.

The RCi for desloratadine production and loratadine disappearanc e was 13 and 26% respectively for CYP 2D6 and 63 and 114% respectively for CYP 3A4. The reason for this unrealistic CYP 3A4 rate contribution of over 100% is unclear; however, it can be attributed to some limitations of the RAF approach. According to Xue-Qing Li, the RAF may suffer from its assumption that the factors that affect the metabolism of enzyme mark er reaction are similar to those of the test compound, which is false for s ome test compounds and some enzymes (Xue-Qing et al., 2003). Despite this limitation, this result confirms that CYP 3A4 is the major isoform involved in the overall metabolic clearance of loratadine consumption.

Ketoconazole, a typical inhibitor of CYP 3A4 (Back et al., 1989, 1992; Pichard et al., 1990) had a strong inhibitory effect on the metabolism of loratadine and decreased desloratadine formation by 66.4%, confirming further that loratadine is mainly metabolised by CYP3A4 in HLM and that desloratadine is mainly produc ed through this pathway. Quinidine, a selective inhibitor of CYP 2D6 (Cholerton et al., 1992), also inhibited desloratadine formation in a concentration -dependent manner, with maximum inhibition of 33%. The maximal inhibition of loratadine consumption by quinidine (10 µM) was 16.5%.

A previous *in vitro* study on loratadine metabolism demonstrated that desloratadine formation by HLM was highly correlated with testosterone 6ß -hy droxylation, a CYP 3A mediated reaction (Yumibe et al., 1996), suggesting that loratadine was metabolised to desloratadine primarily by CYP 3A4 (Yumibe et al., 1996). However, by incubating loratadine with various cDNA-expressed human microsomes, the catalytic formation rate was shown to be approximately 5-fold greater in c DNA -expressed CYP 2D6 than CYP 3A4 microsomes (Yumibe et al., 1996). However, inhibition studies conducted by the same team showed that quinidine at a concentration of 5 µM inhibited desloratadine formation by less than 20% and that troleandomycin, a CYP 3A4 inhibitor, inhibited the rate of desloratadine formation by 75% (Yumibe et al., 1996). The present study clearly alleviates this discrepancy by consistently showing that CYP 3A4 is the main isoform involved in both loratadine metabolism and desloratadine production.

The identification of the enzymes involved in the metabolism of loratadine may be useful for the prediction, or understanding, of the potential interaction of loratadine with other drugs metabolised by CYP 3A4 or CYP 2D6, as these two enzymes metabolise a broad spectrum of drugs. For instance, increased exposure to loratadine was obs erved after concomitant administration of nefazodone (a phenylpiperazine antidepressant) at therapeutic dose in healthy subjects, due to CYP3A inhibition by nefazodone, and this was associated with QTc prolongation on the electrocardiogram, a risk factor of ventricular arrhythmia and sudden death (Darrell et al., 2001).

Further research is also warranted for a more complete understanding of the influence of CYP 2D6 and CYP 3A4 polymorphisms on loratadine metabolism. The technological evolution of modern mass analysers has greatly contributed to impose LC-MS/MS as one of the most powerful analytical tools in the field of xenobiotics metabolism studies (Sauvage et al., 2006; Shuguang et al., 2006), for both qualitative (metabolite characterization) and quantitative (kinetics) information (Marquet, 2002). In the present paper, turbulent-flow chromatography-mass spectrometry (TFC-MS/MS) allowed for the completion of many experiments to investigate lorat adine metabolism pathways and kinetics *in vitro*, while in the first part of this study, a general unknown screening LC-MS/MS technique based on a hybrid linear ion-trap-triple quadrupole mass spectrometer allowed for the specific and fast detection of nineteen loratadine metabolites.

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