A Long-Standing Mystery Solved: The Formation of 3-Hydroxydesloratadine Is Catalyzed by CYP2C8 But Prior Glucuronidation of Desloratadine by UDP-Glucuronosyltransferase 2B10 Is an Obligatory Requirement

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ABSTRACT

Desloratadine (Clarinex), the major active metabolite of loratadine (Claritin), is a non-sedating long-lasting antihistamine that is widely used for the treatment of seasonal allergic rhinitis and chronic idiopathic urticaria. For over 20 years, it has remained a mystery as to which enzymes are responsible for the formation of 3-hydroxydesloratadine, the major active human metabolite, largely due to the inability of any in vitro system tested thus far to generate this metabolite. In this study, we demonstrated that cryopreserved human hepatocytes (CHHs) form 3-hydroxydesloratadine and its corresponding O-glucuronide. CHHs catalyzed the formation of 3-hydroxydesloratadine with a $K_m$ of 1.6 $\mu$M and a $V_{max}$ of 1.3 pmol/min per million cells. Chemical inhibition of cytochrome P450 (P450) enzymes in CHHs demonstrated that gemfibrozil glucuronide (CYP2C8 inhibitor) and 1-aminobenzotriazole (general P450 inhibitor) inhibited 3-hydroxydesloratadine formation by 91% and 98%, respectively. Other inhibitors of CYP2C8 (gemfibrozil, montelukast, clopidogrel glucuronide, repaglinide, and cerivastatin) also caused extensive inhibition of 3-hydroxydesloratadine formation (73%–100%). Assessment of desloratadine, amiodarone, and paclitaxel metabolism by a panel of individual CHHs demonstrated that CYP2C8 marker activity robustly correlated with 3-hydroxydesloratadine formation ($r^2$ of 0.70–0.90). Detailed mechanistic studies with sonicated or saponin-treated CHHs, human liver microsomes, and S9 fractions showed that both NADPH and UDP-glucuronic acid are required for 3-hydroxydesloratadine formation, and studies with recombinant UDP-glucuronosyltransferase (UGT) and P450 enzymes implicated the specific involvement of UGT2B10 in addition to CYP2C8. Overall, our results demonstrate for the first time that desloratadine glucuronidation by UGT2B10 followed by CYP2C8 oxidation and a deconjugation event are responsible for the formation of 3-hydroxydesloratadine.

INTRODUCTION

Desloratadine (Clarinex) is a second-generation, non-sedating selective H1-receptor histamine antagonist with long-acting activity and is widely used for the treatment of seasonal allergic rhinitis and chronic idiopathic urticaria. Desloratadine is also the major active metabolite of the antihistamine loratadine (Claritin) and has a half-life of 21–27 hours with moderate plasma protein binding (82%–87%) permitting once-daily dosing (Henz, 2001; Molnard et al., 2004; Devillier et al., 2008). The major in vivo human active metabolite of desloratadine is 3-hydroxydesloratadine, which is subsequently glucuronidated to 3-hydroxydesloratadine O-glucuronide. Both are excreted in roughly equal amounts in urine and feces (Ramanathan et al., 2007). Furthermore, 3-hydroxydesloratadine and its glucuronide were found to be major metabolites in humans, but only trace levels were detectable in nonclinical species such as mice, rats, and monkeys (Ramanathan et al., 2006), leading to the concern that nonclinical species may not have been adequately exposed to these metabolites in safety studies.

The conversion of loratadine to desloratadine (a dealkylation reaction leading to loss of a descarboethoxyl moiety) was previously shown to be catalyzed by CYP3A4 and, to a lesser extent, by CYP2D6 (Yumibe et al., 1995, 1996; Dridi and Marquet, 2013). However, the enzymology surrounding the conversion of desloratadine to 3-hydroxydesloratadine has remained a mystery both prior to and since its approval by the U.S. Food and Drug Administration (FDA) in 2001 (Clarinex label; http://www.accessdata.fda.gov/drugsatfda_docs/label/2001/21165b1l.pdf). Ghosal et al. (2009) examined the metabolism of loratadine and further characterized it in the in vitro enzymology of the metabolites using pooled human liver microsomes (HLMs) and recombinant cytochrome P450 (P450) enzymes, demonstrating that desloratadine, 5-hydroxydesloratadine, and 6-hydroxydesloratadine formation could be mediated by CYP3A4, CYP2D6, and CYP2C19. However, they were unable to detect 3-hydroxydesloratadine in either in vitro test system and therefore were unable to identify which enzyme or enzymes were involved in its formation. However, the subsequent conjugation of 3-hydroxydesloratadine to 3-hydroxydesloratadine-O-glucuronide was previously shown to be catalyzed in vitro by recombinant UDP-glucuronosyltransferases UGT1A1, UGT1A3, and UGT2B15 (Ghosal et al., 2004).

Clinical pharmacology and safety studies demonstrated that some individuals have a phenotypic polymorphism in the metabolism of desloratadine with greatly reduced formation of 3-hydroxydesloratadine, resulting in a 3-hydroxydesloratadine/desloratadine exposure ratio of

ABBREVIATIONS: 1-ABT, 1-aminobenzotriazole; CHH, cryopreserved human hepatocyte; FDA, U.S. Food and Drug Administration; FMO, flavin-containing monooxygenase; HLM, human liver microsome; HS9, human liver S9 fraction; LC-MS/MS, liquid chromatography tandem mass spectrometry; MRM, multiple reaction monitoring; P450, cytochrome P450; UGT, UDP-glucuronosyltransferase.
<0.1 or a desloratadine half-life of >50 hours (Prenner et al., 2006). These poor metabolizers of desloratadine were found to have a general population frequency of 6% and were most frequent in the African-American population (17%) compared with Caucasian (2%), Native-American (8%), Hispanic (2%), and Jordanian populations (3%) (Prenner et al., 2006; Hakooz and Salem, 2012). Exposure to desloratadine in poor metabolizers resulted in a 6-fold increase in the desloratadine area under the curve compared with extensive metabolizers, leaving the FDA unable to rule out an increased risk of adverse events in poor metabolizers (Clarinetx label; http://www.accessdata.fda.gov/drugsatfda_docs/label/2001/21165lbl.pdf). Because the enzymology of 3-hydroxydesloratadine formation has not been elucidated, the genetic basis for the poor metabolizer phenotype has not been determined.

In this study, we sought to identify the enzyme or enzymes responsible for the formation of 3-hydroxydesloratadine by identifying an in vitro test system capable of generating the metabolite. In this report, we demonstrate that 3-hydroxydesloratadine can be formed in cryopreserved human hepatocytes (CHHs). Using reaction phenotyping approaches (correlation analysis, chemical inhibition, and studies with recombinant enzymes), we elucidated the main human metabolic enzymes responsible for converting desloratadine to 3-hydroxydesloratadine.

We established that the 3-hydroxylation of desloratadine is catalyzed by CYP2C8, but the reaction is unusual because the prior glucoronidation of desloratadine by UGT2B10 is an obligatory step in the reaction.

### Materials and Methods

**Chemicals and Reagents.** 1-Aminobenzotriazolone (1-ABT), desloratadine, furafylline, gemfibrozil, ketoconazole, mibefradil, paclitaxel, paroxetine, phenycyclidine, quinidine, and repaglinide were purchased from Sigma-Aldrich (St. Louis, MO). Cerivastatin, CYP3cide, esomeprazole, and gemfibrozil glucuronide were purchased from Toronto Research Chemicals (Toronto, ON, Canada). Montelukast was purchased from Sequoia Research Products (Pangbourne, UK). Tienilic acid was purchased from Cypex (Dundee, Scotland, UK). Amodiaquine and troleandomycin were purchased from US Pharmacopeia (Rockville, MD). 3-Hydroxydesloratadine and clopidogrel glucuronide were purchased from Santa Cruz Biotechnology (Dallas, TX). 3-Hydroxydesloratadine glucuronide, 5-hydroxydesloratadine, and 6-hydroxydesloratadine were purchased from TLC PharmaChem (Vaughan, ON, Canada). 3-Hydroxydesloratadine-d4 was purchased from Medical Isotopes, Inc. (Pelham, NH). The sources of all other reagents were described previously (Ogilvie et al., 2006; Parkinson et al., 2011; Kazmi et al., 2014a).

**Test System.** Pooled HLMs (n = 200, mixed sex), pooled human liver S9 mixes (n = 50 or 100, mixed sex) or individual donor CHHs (see Supplemental Table 1 for donor information) were prepared from nontransplantable livers and characterized at XenoTech, LLC (Lenexa, KS) as previously described (Pearce et al., 1996; Parkinson et al., 2004). Hepatocytes from Sprague-Dawley rats (male, n = 4), Beagle dogs (male, n = 3), CD1 mice (male, n = 7), Rhesus monkeys (male, n = 3), New Zealand white rabbits (male, n = 3), and Gottingen minipigs (male, n = 3) were prepared and characterized at XenoTech, LLC as previously described.

**In Vitro Incubations of Desloratadine with HLMs, HS9s, and CHHs.** Desloratadine was incubated with HLMs, HS9s, and CHHs to determine whether any of these in vitro test systems would support the formation of 3-hydroxydesloratadine. Briefly, 1 or 10 μM desloratadine was incubated at 37°C in 200-μl incubation mixtures containing pooled HLMs (0.1 or 1 mg/ml) or HS9s (0.5 or 5 mg/ml), potassium phosphate buffer (50 mM, pH 7.4), MgCl2 (3 mM), EDTA (1 mM, pH 7.4), a NADPH-generating system (consisting of 1 mM NADP, 5 mM glucose-6-phosphate, and 1 U/ml glucose-6-phosphate dehydrogenase) for 0, 0.5, 1, 2, 4, and 4 hours. For assays with pooled CHHs (n = 50), desloratadine incubations were conducted at 37°C with 95% humidity and 5% CO2 on an orbital shaker (approximately 150 rpm) in 160-μl incubation mixtures containing pooled CHHs (1 million cells/ml) and Williams’ E media supplemented with 2 mM glutaMAX (Gibco, Grand Island, NY) and 0.1 mM HEPES. Reactions were initiated by the addition of an NADPH-generating system (for HLMs and HS9s) or hepatocytes (for CHH assays) and terminated by the addition of 200 μl acetonitrile (160 μl for CHH assays) containing 3-hydroxydesloratadine-d4 as an internal standard. Precipitated protein was removed by centrifugation (920g for 10 minutes at 10°C) followed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis as described below.

### Correlation Analysis of CHHs with a Range of CYP2C8 Activities.

Individual donor CHHs that were precharacterized with a range of CYP2C8 activities were assessed for 3-hydroxydesloratadine formation. Briefly, nine individual lots of hepatocytes were incubated at 1 million cells/ml with 1 or 10 μM desloratadine, amodiaquine, or paclitaxel in 160-μl incubation mixtures
containing Williams’ E media supplemented with 2 mM glutaMAX (Gibco) and 0.1 mM HEPES. Reactions were initiated by the addition of hepatocytes and conducted for 10 minutes (amodiaquine), 30 minutes (paclitaxel), or 2 hours (desloratadine) at 37°C with 95% humidity and 5% CO2 in an orbital shaker (approximately 150 rpm). Reactions were terminated by the addition of an equal volume of acetonitrile containing internal standard, followed by protein precipitation and LC-MS/MS analysis.

Exogenous Cofactor Addition with CHHs, HLMs, or HS9s. Pooled CHHs (n = 100; 1 million cells/ml) were treated with 0.01% (w/v) saponin (5 minutes) or disrupted with a probe sonicator (45 seconds at 40–60% amplitude) followed by incubation in 100-μl incubation mixtures containing 10 μM desloratadine in Williams’ E media supplemented with 2 mM glutaMAX (Gibco) and 0.1 mM HEPES. Incubations were conducted in the presence or absence 0.1 mM NADPH and/or 1 mM UDP-GlcUA and conducted for 2 hours at 37°C with 95% humidity and 5% CO2 on an orbital shaker (approximately 150 rpm). For incubations with pooled subcellular fractions, HLMs at 0.1 and 1 mg/ml or HS9s at 0.5 and 5 mg/ml were pretreated for 15 minutes on ice with 25 μg/ml amelanchin followed by incubation with or without 1 mM chemical NADPH and/or 10 mM UDP-GlcUA at 37°C in 200-μl incubation mixtures containing potassium phosphate buffer (50 mM, pH 7.4), MgCl2 (3 mM), and EDTA (1 mM, pH 7.4) for 0, 1, 2, 4, and 6 hours. Other cofactors such as NADH, FAD, AMP, and ATP were also tested (each at 1 mM). All reactions were terminated by the addition of an equal volume of acetonitrile containing internal standard, followed by protein precipitation and LC-MS/MS analysis.

Recombinant UGT Panel with Recombinant CYP2C8. Recombinant human UGT enzymes—namely, UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT2B4, UGT2B7, UGT2B10, UGT1A15, and UGT2B17 Superomes (Corning)—were evaluated for their ability to form 3-hydroxydesloratadine. Briefly, 0.125 mg/ml recombinant UGT was supplemented with 25 pmol/ml of recombinant CYP2C8, followed by addition of 1 or 10 μM desloratadine and incubation with 1 mM chemical NADPH and 10 mM UDP-GlcUA at 37°C in 200-μl incubation mixtures containing potassium phosphate buffer (50 mM, pH 7.4), MgCl2 (3 mM), and EDTA (0.5 or 1 mM, pH 7.4) for 2 hours. Reactions were terminated by the addition of an equal volume of acetonitrile containing internal standard, followed by protein precipitation and LC-MS/MS analysis.

Analytical Methods. Samples were analyzed by LC-MS/MS using a method developed in-house. The liquid chromatography system comprised a Shimadzu DGU-20A3 degasser (Shimadzu, Columbia, MD). A liquid chromatography gradient employing 0.2% formic acid in water (A) and acetonitrile (B) at 30 psi, the source temperature was 600°C and conducted for 6-hydroxydesloratadine (4.7 minutes), and 503/327 for 3-hydroxydesloratadine glucuronide (5.1 minutes). Retention times for these five analytes were confirmed by comparison with reference standards. The electrospray voltage was 3.5 kV. The product ion spectra were obtained using positive mode and electrospray ionization. A multiple reaction monitoring (MRM) information-dependent acquisition detection method was developed based on manually derived transitions for known and predicted metabolites of desloratadine. Twenty-seven transitions for known and predicted metabolites and oxidative metabolite transitions and 30 V for labile conjugated metabolite transitions were evaluated for their ability to form 3-hydroxydesloratadine. Briefly, 0.125 mg/ml recombinant UGT was supplemented with 25 pmol/ml of recombinant CYP2C8, followed by addition of 1 or 10 μM desloratadine and incubation with 1 mM chemical NADPH and 10 mM UDP-GlcUA at 37°C in 200-μl incubation mixtures containing potassium phosphate buffer (50 mM, pH 7.4), MgCl2 (3 mM), and EDTA (0.5 or 1 mM, pH 7.4) for 2 hours. Reactions were terminated by the addition of an equal volume of acetonitrile containing internal standard, followed by protein precipitation and LC-MS/MS analysis.

Determination of an In Vitro Test System Capable of Producing 3-Hydroxydesloratadine. To determine whether 3-hydroxydesloratadine could be formed in any conventional in vitro test system, desloradine (1 and 10 μM) was incubated with HLMs (0.1 and 1 mg/ml), HS9s (0.5 and 5 mg/ml), and CHHs (1 million cells/ml) in a time course experiment up to 4 hours as described in Materials and Methods. The results for 3-hydroxydesloratadine formation with 1 μM desloradine are shown in Fig. 1 (10 μM desloradine data were similar and not shown). 3-Hydroxydesloratadine formation was not observed in any HLM or HS9 sample, consistent with previous reports (Ghosal et al., 2009). However, 3-hydroxydesloradine was detected in CHHs as early as 30 minutes, with linear metabolite formation up to 4 hours. Furthermore, 3-hydroxydesloradine glucuronide was also detected in CHHs as early as 1 hour with 10 μM desloradine, but only at 4 hours with 1 μM desloradine. Additional expected hydroxylated metabolites such as 5-hydroxydesloradine and 6-hydroxydesloradine were detected in all three test systems.

Assessment of the Kinetics of 3-Hydroxydesloratadine Formation. Having established that CHHs were the only test system capable of forming 3-hydroxydesloradine, we sought to determine the Km and Vmax of 3-hydroxydesloradine formation from desloradine. Linearity of 3-hydroxydesloradine formation was established beyond 2 hours. Desloradine was incubated at nine concentrations with pooled CHHs (1 million cells/ml) for 2 hours as described in Materials and Methods. As shown in Fig. 2, 3-hydroxydesloradine formation followed Michaelis–Menten kinetics, with Km and Vmax values of 1.6 μM and 1.3 pmol/min per million cells, respectively.

Assessment of the Species Specificity of 3-Hydroxydesloratadine Formation. We evaluated whether 3-hydroxydesloradine could be formed by hepatocytes from different species. Mouse, rat, rabbit, dog, minipig, monkey, and human hepatocytes (1 million cells/ml) were incubated with 1 or 10 μM desloradine for 2 hours as described in Materials and Methods. The results for 3-hydroxydesloradine formation (Fig. 3) showed that at 1 μM desloradine, rabbit and
human hepatocytes formed similar amounts of 3-hydroxydesloratadine, whereas dog hepatocytes formed one-third as much and monkey hepatocytes formed only a trace amount. By contrast, no 3-hydroxydesloratadine was detected in incubations of 1 μM desloratadine with mouse, rat, or minipig hepatocytes. At 10 μM desloratadine, only rabbit and human hepatocytes formed 3-hydroxydesloratadine, with rabbit hepatocytes forming three times as much 3-hydroxydesloratadine as human hepatocytes. No 3-hydroxydesloratadine formation was observed in incubations of 10 μM desloratadine with mouse, rat, dog, minipig, or monkey hepatocytes. Formation of both 5-hydroxydesloratadine and 6-hydroxydesloratadine in each species was determined simultaneously (shown in Supplemental Fig. 1), with all animal species forming higher levels of these metabolites than human hepatocytes. Rabbit hepatocytes formed the greatest amount of 5-hydroxydesloratadine, whereas rabbit and minipig hepatocytes formed the greatest amounts of 6-hydroxydesloratadine.

Identification of the Enzyme Responsible for 3-Hydroxydesloratadine Formation Using Recombinant Enzymes. To determine which specific drug-metabolizing enzymes were responsible for the formation of 3-hydroxydesloratadine, recombinant P450 and FMO enzymes (50 pmol/ml) were incubated with 1 and 10 μM desloratadine and incubated for 1 hour as described in Materials and Methods. The data are summarized in Table 1. No 3-hydroxydesloratadine was detected in any recombinant P450 or FMO enzyme sample tested, consistent with previously reported findings (Ghosal et al., 2009). Both 5-hydroxydesloratadine and 6-hydroxydesloratadine were readily formed by CYP1A1, CYP2D6, and CYP3A4, with trace metabolic formation observed for several other P450 enzymes (Table 1). A time course experiment for up to 6 hours with recombinant CYP2C8 also failed to generate any 3-hydroxydesloratadine (data not shown).

Identification of the Enzyme Responsible for 3-Hydroxydesloratadine Formation Using Chemical Inhibitors. Since a recombinant P450/FMO enzyme approach was unable to identify any enzyme capable of forming 3-hydroxydesloratadine, we evaluated the effects of P450-selective inhibitors on the formation of 3-hydroxydesloratadine by CHHs. Initially, a panel of chemical inhibitors specific to different P450, with trace metabolite formation observed for several other P450 enzymes (Table 1). A time course experiment for up to 6 hours with recombinant CYP2C8 also failed to generate any 3-hydroxydesloratadine (data not shown).

Correlation of 3-Hydroxydesloratadine Formation with Known CYP2C8 Activities. Hepatocytes from nine individual human donors with a range of CYP2C8 activity toward amodiaquine and paclitaxel were assessed for their ability to form 3-hydroxydesloratadine, as described in Materials and Methods. As shown in Fig. 5, the sample-to-sample variation in the 3-hydroxylation of desloratadine (1 and 10 μM) correlated well with the N-dealkylation of amodiaquine (1 and 10 μM), with r² values of 0.84 and 0.90 (at 1 and 10 μM), and with the N-dealkylation of amodiaquine, with r² values of 0.84 and 0.70 (at 1 and 10 μM). As expected, amodiaquine and paclitaxel activities highly correlated with each other, with r² values of 0.77 and 0.80 (at 1 and 10 μM), as shown in Supplemental Fig. 3.

Determining the Reason Why 3-Hydroxydesloratadine Forms in Hepatocytes But Not in Subcellular Fractions. We examined the role of cell integrity and various cofactors involved in xenobiotic metabolism as a first approach to understand why CYP2C8 in CHHs could convert desloratadine to 3-hydroxydesloratadine, whereas HLMs, H9Ns, and recombinant P450s could not. As described in Materials and Methods, CHHs were treated with 0.01% saponin (to permeabilize the plasma membrane) or sonication (to completely disrupt the plasma membrane) in media supplemented with 10 μM desloratadine and various cofactors such as NADPH, NADH, FAD, AMP, ATP, and UDP-GlcUA. As shown in Fig. 6A, when intact CHHs were treated with 0.01% saponin, the formation of 3-hydroxydesloratadine was reduced by 90%. Addition of exogenous

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**Fig. 2.** Determination of the enzyme kinetics for the formation of 3-hydroxydesloratadine from desloratadine in CHHs. As described in Materials and Methods, the kinetics for the formation of 3-hydroxydesloratadine were determined in CHHs (1 million cells/ml) with 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, and 30 μM desloratadine incubated for 2 hours. The left panel represents rate versus substrate concentration, and the right panel represents the Eadie–Hofstee plot.
NADPH did not change the rate of 3-hydroxydesloratadine formation; however, addition of UDP-GlcUA and NADPH plus UDP-GlcUA partially restored 3-hydroxydesloratadine formation to 22% and 37% of that observed in intact CHHs, respectively. Similarly, when CHHs were probe sonicated, 3-hydroxydesloratadine formation was almost completely eliminated with only 2.5% activity remaining. Addition of exogenous NADPH here also did not alter the level of 3-hydroxydesloratadine formation; however, addition of UDP-GlcUA and NADPH plus UDP-GlcUA again partially restored 3-hydroxydesloratadine formation to 15% and 34% of that observed in intact CHHs, respectively. To ascertain whether a similar combination of cofactors could confer desloratadine 3-hydroxylase activity on subcellular fractions, HLMs (0.1 and 1 mg/ml) and HS9s (0.5 and 5 mg/ml) were incubated with 10 μM desloratadine for up to 6 hours with NAPDH and/or UDP-GlcUA, as described in Materials and Methods. With either of these subcellular fractions, only the addition of a combination of NADPH plus UDP-GlcUA resulted in 3-hydroxydesloratadine formation; addition of NADPH alone or UDP-GlcUA alone did not. Representative data from 1 mg/ml HLM and 5 mg/ml HS9 are shown in Fig. 6B (0.1 mg/ml HLM and 0.5 mg/ml HS9 data were similar and are shown in Supplemental Fig. 4). The addition of several other cofactors (NADH, FAD, AMP, and ATP) had no effect on the formation of 3-hydroxydesloratadine in sonicated or permeabilized CHHs or in HLMs or HS9s (data not shown).

Identification of the UGT Enzymes Involved in 3-Hydroxydesloratadine Formation. Having established that a combination of both UDP-GlcUA and NADPH was necessary to support 3-hydroxydesloratadine formation by disrupted CHHs or subcellular fractions, we sought to determine the specific UGT enzyme involved in 3-hydroxydesloratadine formation. As described in Materials and Methods, a panel of 13 recombinant UGT enzymes (at 0.125 mg/ml each) supplemented with recombinant CYP2C8 (25 pmol/ml) was incubated for 2 hours with 1 or 10 μM desloratadine (Fig. 3).

### TABLE 1

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3-OH, 3-hydroxydesloratadine; 5-OH, 5-hydroxydesloratadine; 6-OH, 6-hydroxydesloratadine; NA, not applicable.

*Data from 10-μM desloratadine experiments.

*Trace levels detected for CYP1A2, CYP1B1, CYP1B2, CYP2B6, CYP2B8, CYP2C8, CYP2C18, CYP2C19, CYP2J2, CYP3A4, and CYP3A5 with 1 μM desloratadine.
in the presence of both NADPH with UDP-GlcUA. As shown in Fig. 7, 3-hydroxydesloratadine was formed by a combination of UGT2B10 and CYP2C8. Formation of 3-hydroxydesloratadine was not observed when CYP2C8 was incubated with any other recombinant UGT enzyme.

Discussion

Loratadine (Claritin), first introduced to the U.S. market in 1993, is metabolized primarily by CYP3A4 and CYP2D6 to its pharmacologically active major metabolite desloratadine (Yumibe et al., 1995, 1996; Dridi and Marquet, 2013). As a drug in its own right, desloratadine (Clarinex in the United States, and Aerius in Europe) received FDA approval in 2001. It has long been known that the major circulating metabolite of desloratadine in humans is 3-hydroxydesloratadine, formed by hydroxylation of the pyridine ring (Clarinex label; http://www.accessdata.fda.gov/drugsatfda_docs/label/2001/21165lbl.pdf). However, as acknowledged in the package insert, the enzyme or enzymes responsible for forming 3-hydroxydesloratadine were unknown when desloratadine was approved by the FDA in 2001, and have remained unknown since that time. Although presumably formed by P450, conventional in vitro test systems do not convert desloratadine to the 3-hydroxy metabolite (Ghosal et al., 2009). In this study, we confirmed that HLMs, HS9s, and recombinant human P450 enzymes all failed to convert desloratadine to 3-hydroxydesloratadine, but we demonstrated for the first time that CHHs are capable of forming the 3-hydroxy metabolite (Fig. 1; Table 1). In vitro formation of this previously elusive metabolite allowed us to investigate the enzymology surrounding its formation. The formation of 3-hydroxydesloratadine by CHHs conformed to simple Michaelis–Menten kinetics with a $V_{\text{max}}$ of 1.26 pmol/min per million cells and a $K_m$ of 1.57 $\text{mM}$ (Fig. 2), which is consistent with the reported plasma $C_{\text{max}}$ of desloratadine in humans (1.3 $\mu$M) (Clarinex label; http://www.accessdata.fda.gov/drugsatfda_docs/label/2001/21165lbl.pdf). These results strongly suggest that hepatic metabolism is responsible for the formation of 3-hydroxydesloratadine and are consistent with the urinary and biliary excretion of this metabolite (and its O-glucuronide) in humans (Ramanathan et al., 2006, 2007). Data on the metabolism of desloratadine in nonclinical species are limited, with 5-hydroxydesloratadine and 6-hydroxydesloratadine reported as the major excreted metabolites in mice, rats, and monkeys. In contrast with the situation in humans, 3-hydroxydesloratadine was found to be a minor or trace plasma, urinary, and fecal metabolite in nonclinical species (Ramanathan et al., 2006, 2007).
In our assessment of desloratadine metabolism in hepatocytes from different species (Fig. 3), 3-hydroxydesloratadine was observed at low levels in mouse, rat, and monkey hepatocytes, consistent with previously reported in vivo findings (Ramanathan et al., 2006). However, rabbits, dogs, and humans were able to form the 3-hydroxy metabolite in incubations at a pharmacologically relevant concentration (1 μM desloratadine), whereas only rabbits and humans formed it at the high concentration (10 μM desloratadine).

It is unclear whether rabbits were evaluated as nonclinical metabolism species during desloratadine development; however, our data suggest they may be appropriate species to model 3-hydroxydesloratadine exposure. Formation of 5-hydroxydesloratadine and 6-hydroxydesloratadine was faster in hepatocytes from all nonclinical species tested compared with human hepatocytes (Supplemental Fig. 1), consistent with the in vivo data (Ramanathan et al., 2005, 2006). In this study, formation of 5-hydroxydesloratadine and 6-hydroxydesloratadine was faster in hepatocytes from all nonclinical species tested compared with human hepatocytes (Supplemental Fig. 1), consistent with the in vivo findings (Ramanathan et al., 2006). However, rabbits, dogs, and humans were able to form the 3-hydroxy metabolite in incubations at a pharmacologically relevant concentration (1 μM desloratadine), whereas only rabbits and humans formed it at the high concentration (10 μM desloratadine).

The nonspecific inhibitor 1-ABT markedly inhibited (98%) the formation of 3-hydroxydesloratadine by CHHs, confirming expectations that this reaction is catalyzed by P450 (Fig. 4A). Marked inhibition (91%) was also observed with gemfibrozil glucuronide (Fig. 4A). Gemfibrozil glucuronide is an irreversible (mechanism-based) inhibitor of CYP2C8 and is widely used as an in vitro diagnostic inhibitor of this enzyme (Ogilvie et al., 2006; Parkinson et al., 2011; Kazmi et al., 2014b). To confirm CYP2C8 involvement in 3-hydroxydesloratadine formation, a panel of known CYP2C8 inhibitors or substrates (competitive inhibitors) was evaluated (Fig. 4B)—namely, montelukast, repaglinide, cerivastatin, clopidogrel glucuronide, and both gemfibrozil and gemfibrozil glucuronide (Bidstrup et al., 2003; Walsky et al., 2005; Ogilvie et al., 2006; Tornio et al., 2014). Strong inhibition of 3-hydroxydesloratadine formation was observed with all CYP2C8 inhibitors and correlated well with the degree of inhibition in the metabolism of two CYP2C8 substrates (paclitaxel and amodiaquine), supporting CYP2C8 as the P450 enzyme responsible for 3-hydroxydesloratadine formation. A comparison of CYP2C8 activity in nine individual samples of human hepatocytes demonstrated high correlation ($r^2 = 0.7–0.9$) between 3-hydroxydesloratadine formation and both amodiaquine $N$-dealkylation and paclitaxel 6-$\alpha$-hydroxylation (Fig. 5).

The results presented thus far seem paradoxical. They raise the following question: If CYP2C8 is the major enzyme responsible for converting desloratadine to 3-hydroxydesloratadine in human hepatocytes, based on chemical inhibition and correlation analysis, why is no 3-hydroxydesloratadine formed by HLMs, HS9s, or recombinant CYP2C8? We hypothesized that perhaps cellular integrity or the presence of specific cofactors was critical for 3-hydroxydesloratadine formation. In support of this possibility, we found that permeabilizing the plasma membrane of hepatocytes with saponin or completely disrupting the membrane by sonication greatly reduced 3-hydroxydesloratadine formation (Fig. 6A). Addition of various cofactors to permeabilized/sonicated hepatocytes revealed that formation of 3-hydroxydesloratadine could be partially restored by the addition of both NAPDH and UDP-GlcUA. Modest recovery was also observed in permeabilized/sonicated hepatocytes supplemented with only UDP-GlcUA presumably because there was sufficient endogenous NADPH to support some 3-hydroxy metabolite formation. Subsequent experiments with HLMs and HS9s (Fig. 6B) confirmed the requirement of both NADPH and UDP-GlcUA for the formation of 3-hydroxydesloratadine.
These results suggested that, in addition to oxidation by CYP2C8, glucuronidation plays a key role in the formation of 3-hydroxydesloratadine. To further explore this possibility, desloratadine was incubated with recombinant CYP2C8 in the absence or presence of a panel of recombinant UGT enzymes (with NADPH and UDP-GlcUA as cofactors). In the absence of any UGT enzyme, CYP2C8 did not form 3-hydroxydesloratadine but did so in the presence of UGT2B10 (Fig. 7). These results suggest that desloratadine is glucuronidated by UGT2B10 and that desloratadine glucuronide, not desloratadine itself, is the substrate that undergoes 3-hydroxylation by CYP2C8. Furthermore, the results suggest that the glucuronide moiety introduced by UGT2B10 is cleaved during or shortly after metabolism by CYP2C8.

A proposed metabolic scheme for 3-hydroxydesloratadine formation is shown in Fig. 8. The first step is proposed as formation of desloratadine N-glucuronide by UGT2B10, followed by hydroxylation to 3-hydroxydesloratadine N-glucuronide by CYP2C8, with subsequent deconjugation to 3-hydroxydesloratadine. Efforts to isolate and characterize the proposed intermediary metabolites are currently underway. An N-glucuronide is proposed as the initial metabolite because there are no hydroxyl or thiol groups available for direct conjugation. UGT2B10 is one of two enzymes, the other being UGT1A4, renowned for their ability to catalyze the N-glucuronidation of drugs, with UGT2B10 being a high-affinity/low-capacity enzyme and UGT1A4 being a low-affinity/high-capacity enzyme (Zhou et al., 2010; Parkinson et al., 2013). Ketotifen, a structural analog of desloratadine, is known to be N-glucuronidated at the piperidine ring to a quaternary N-glucuronide by UGT2B10 and UGT1A4. Furthermore, N-glucuronidation of ketotifen is a prominent reaction in rabbits.

Fig. 6. Formation of 3-hydroxydesloratadine in saponin-treated or sonicated CHHs followed by addition of NADPH and/or UDP-GlcUA. (A) CHHs (1 million cells/ml) were either pretreated with 0.01% saponin or probe sonicated followed by addition of 0.1 mM NADPH and/or 1 mM UDP-GlcUA and incubation with 10 μM desloratadine for 2 hours. (B) Formation of 3-hydroxydesloratadine in subcellular fractions—namely, HLMs (1 mg/ml) and HS9s (5 mg/ml) as assessed over 6 hours with or without 1 mM NADPH and/or 10 mM UDP-GlcUA.

Fig. 7. Assessment of 3-hydroxydesloratadine formation with a panel of recombinant UGT enzymes supplemented with recombinant CYP2C8. Thirteen recombinant UGT enzymes (at 0.125 mg/ml) were assessed for their ability to form 3-hydroxydesloratadine when supplemented with recombinant CYP2C8 (25 pmol/ml) and 1 mM NADPH with 10 mM UDP-GlcUA, followed by a 2-hour incubation with 1 or 10 μM desloratadine.

Fig. 8. Proposed scheme for the formation of 3-hydroxydesloratadine.
and humans, the two species whose hepatocytes catalyzed the highest rate of formation of 3-hydroxydesloratadine (Kato et al., 2013; Bolleddula et al., 2014). It was previously reported that rabbits may be a particularly useful species for nonclinical studies of drugs that undergo \(N\)-glucuronidation in humans (Chiu and Huskey, 1998). However, \(N\)-glucuronidation by UGT2B10 on the pyridine moiety of desloratadine cannot be ruled out and has been shown to occur in the case of nicotine and cotinine glucuronidation (Murphy et al., 2014).

The ability of CYP2C8 to metabolize a glucuronide conjugate is well established (Parkinson et al., 2013). For example, whereas the \(4'\)-hydroxylation of diclofenac (parent drug) is catalyzed by CYP2C9, the \(4'\)-hydroxylation of diclofenac acyl glucuronide is catalyzed by CYP2C8 (Kumar et al., 2002). This same pattern—in which the aglycone (typically a small acidic substrate) is not metabolized by CYP2C8 (and in some cases is metabolized by CYP2C9), whereas the glucuronide metabolite (a large acidic substrate) is metabolized by CYP2C8—has been reported for estradiol 17-\(O\)-\(\beta\)-glucuronide and the acyl glucuronide conjugates of naproxen, the peroxisome proliferator-activated receptor \(\alpha\) agonist 2-[[5,7-dipropyl-3-(trifluoromethyl)-1,2-benzisoxazol-6-yl]oxy]-2-methylpropanoic acid, and gemfibrozil (Delaforge et al., 2005; Kochansky et al., 2005; Ogilvie et al., 2006; Parkinson et al., 2013). In the case of gemfibrozil, the CYP2C8-mediated hydroxylation of its 1-\(O\)-\(\beta\)-glucuronide forms a benzyl radical intermediate that causes irreversible inhibition of CYP2C8 (Ogilvie et al., 2006; Baer et al., 2009).

The conversion of desloratadine by UGT2B10 to an \(N\)-glucuronide that is subsequently hydroxylated by CYP2C8 is consistent with the known properties of these enzymes. Nevertheless, the formation of 3-hydroxydesloratadine is unusual because no 3-hydroxylation is detectable in the absence of glucuronidation and because the \(N\)-glucuronide is cleaved during or shortly after CYP2C8-dependent hydroxylation. Interestingly, although 3-hydroxydesloratadine is produced from an \(N\)-glucuronide (formed by UGT2B10 and cleaved after hydroxylation by CYP2C8), 3-hydroxydesloratadine itself is subsequently converted to an \(O\)-glucuronide (at the 3-hydroxy position) by UGT1A1, UGT1A3, and UGT2B15 (Ghosal et al., 2004).

It is unclear whether there is any potential for drug–drug interactions with desloratadine, because it has a large therapeutic safety margin. As a perpetrator, desloratadine has been shown not to be an inhibitor of CYP1A2, CYP2C9, CYP2C19, CYP2D6, or CYP3A4 (Barecki et al., 2001). However, to our knowledge, inhibition of CYP2C8 and UGT2B10 has not been evaluated, so it is unclear whether desloratadine could cause any clinically relevant interactions with substrates of these enzymes. A recent clinical study examining the effect of desloratadine on montelukast serum levels found no significant difference in montelukast serum levels in fixed-dose combination with desloratadine.
The pharmacogenetic basis for the 3-hydroxydesloratadine poor metabolizer phenotype has remained a mystery. It has been reported that the polymorphism surrounding 3-hydroxydesloratadine formation occurs in approximately 6% of the general population and at a frequency of 17% in African Americans, with poor metabolizers having approximately 6-fold greater systemic exposure than extensive metabolizers (Premter et al., 2006). Our results suggest that CYP2C8 and/or UGT2B10 polymorphism may be responsible for the poor metabolizer phenotype. A large number of CYP2C8 genetic polymorphisms have been identified, with CYP2C8*2, CYP2C8*3, CYP2C8*4, CYP2C8*8, and CYP2C8*14 alleles shown to have decreased functional activity (Dai et al., 2001; Bahadur et al., 2002; Hichiya et al., 2005; Gao et al., 2010; Hanioka et al., 2010; Jiang et al., 2011). However, little is currently known about UGT2B10 polymorphisms, although the UGT2B10*2 allele has been shown to correspond to a functional decrease in nicotine and cotinine glucuronide formation (Chen et al., 2007). Further studies will be necessary to establish whether genetic polymorphisms of CYP2C8 and/or UGT2B10 can account for the desloratadine poor metabolizer phenotype.

In summary, the following evidence suggests that the conversion of desloratadine to 3-hydroxydesloratadine is mediated by CYP2C8 in conjunction with UGT2B10. First, the formation of 3-hydroxydesloratadine by human hepatocytes is inhibited by reversible and irreversible inhibitors of CYP2C8. Second, in human hepatocytes, the sample-to-sample variation in 3-hydroxydesloratadine formation correlates with CYP2C8 activity toward amodiaquine and paclitaxel. Third, HLMs and S9 fractions do not form 3-hydroxydesloratadine unless supplemented with both NADPH and UDP-GlcUA. Fourth, recombinant CYP2C8 does not form 3-hydroxydesloratadine unless co- incubated with recombinant UGT2B10 and both NADPH and UDP-GlcUA. Finally, no other pair of recombinant P450 and UGT enzymes convert desloratadine to 3-hydroxydesloratadine.

We were unable to detect either desloratadine N-glucuronide (formed by UGT2B10) or 3-hydroxydesloratadine N-glucuronide (the initial metabolite formed by CYP2C8). These glucuronides appear to be very unstable, which is a characteristic of certain other N-glucuronides (Ciotti et al., 1999). Despite this limitation, the identification of CYP2C8 in combination with UGT2B10 in the formation of 3-hydroxydesloratadine contributes to our understanding of the long-standing mystery surrounding the enymology of 3-hydroxydesloratadine formation in humans, providing a pathway for future investigation of the genetic basis for the desloratadine poor pharmacologic phenotype.
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