Clopidogrel and Gemfibrozil Strongly Inhibit the CYP2C8-Dependent Formation of 3-Hydroxydesloratadine and Increase Desloratadine Exposure In Humans

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ABSTRACT

A recent in vitro study suggested that CYP2C8 is essential in the metabolism of desloratadine, an H1 receptor antagonist. If the proposed biotransformation mechanism takes place in vivo in humans, desloratadine could serve as a selective CYP2C8 probe substrate in drug–drug interaction studies. Glucuronide metabolites of clopidogrel and gemfibrozil act as time-dependent inhibitors of CYP2C8, but they have not been compared clinically. We conducted a randomized crossover study in 11 healthy subjects to characterize the involvement of CYP2C8 in desloratadine metabolism and to compare the CYP2C8 inhibitory strength of clopidogrel (300 and 75 mg on two following days) with that of gemfibrozil (600 mg BID for 5 days). Compared with placebo (control), clopidogrel increased the area under the plasma concentration-time curve (AUC0–t) ratios were 21% (P = 7 × 10−10) and 1.7% (P = 8 × 10−11) of control during the clopidogrel and gemfibrozil phases. Our results confirm that CYP2C8 plays a critical role in the formation of 3-hydroxydesloratadine in humans, making desloratadine a potential CYP2C8 probe substrate. Furthermore, the findings corroborate the previous estimates that clinically relevant doses of clopidogrel cause strong CYP2C8 inhibition, whereas those of gemfibrozil almost completely inactivate the enzyme in humans.

Introduction

Desloratadine is a potent, nonselective H1 histamine receptor antagonist indicated for symptomatic relief of urticaria and allergic rhinitis (https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/021165s017,021306014,021312015,021563s003lbl.pdf; Henz, 2001). It has linear pharmacokinetics in the 5- to 20-mg dose range, is 82%–87% bound to plasma proteins, and has a terminal half-life (t1/2) of about 21–27 hours (Henz, 2001; Murdoch et al., 2003; Molimard et al., 2004). It is extensively metabolized, and its major metabolite is 3-hydroxydesloratadine, which is further glucuronidated to 3-hydroxydesloratadine-O-glucuronide (Molimard et al., 2004). A recent in vitro study suggested a central role for cytochrome P450 2C8 (CYP2C8) in the formation of the 3-hydroxy metabolite, involving initial N-glucuronidation of desloratadine followed by CYP2C8-dependent oxidation of the N-glucuronide and subsequent rapid deconjugation (Fig. 1) (Kazmi et al., 2015a). The proposed CYP2C8-selective metabolism and the benign safety profile could make desloratadine a practical CYP2C8 probe substrate in drug–drug interaction (DDI) studies (Prenner et al., 2006).

The acyl-β-D-glucuronide metabolite of the antiplatelet agent clopidogrel and the 1-O-glucuronide metabolite of the fibric acid derivative gemfibrozil act as mechanism-based inhibitors of CYP2C8 (Ogilvie et al., 2006; Tornio et al., 2014; Backman et al., 2016). Accordingly, in humans, clopidogrel and gemfibrozil have significantly increased the exposure to several CYP2C8 substrates such as cerivastatin, repaglinide, pioglitazone, montelukast, and dasabuvir (Backman et al., 2002, 2016; Niemi et al., 2003; Jaakkola et al., 2005; Karonen et al., 2010; Tornio et al., 2014; Itkonen et al., 2016, 2018, 2019). Recently, both clopidogrel and gemfibrozil were suggested as model CYP2C8 inhibitors for DDI studies in the FDA Draft Guidance for Clinical Drug Interaction Studies (https://www.fda.gov/downloads/drugs/guidances/ucm292362.pdf). The CYP2C8-inhibitory strength of clopidogrel, however, has not been directly compared with that of gemfibrozil in a clinical setting.

Glucuronide metabolites have generally been regarded as relatively stable and physiologically inactive compounds, but owing to the reactivity of acyl glucuronides as well as their ability to act as perpetrators in drug interactions, these presumptions have been challenged by Ogilvie et al. (2006), Regan et al. (2010), Tornio et al. (2014), and Backman et al. (2016). Apart from the ability of CYP2C8 to reactivate glucuronides of gemfibrozil, clopidogrel, and deleobuvir, leading to CYP2C8 inactivation, several

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ABBREVIATIONS: AUC, area under the plasma concentration-time curve; BID, twice daily; CI, confidence interval; Cmax, peak plasma concentration; CV, coefficients of variation; DDI, drug–drug interaction; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LLQ, lower limit of quantification; OATP, organic anion-transporting polypeptide; P450, cytochrome P450; QD, every day; t1/2, terminal half-life; tmax, time to Cmax; UGT, uridine 5′-diphospho-glucuronosyltransferase.
glucuronides of xenobiotics and steroid hormones, including those of diclofenac, licofelone, estradiol, and desloratadine, have been shown to undergo CYP2C8-mediated oxidation in vitro (Kumar et al., 2002; Delaforge et al., 2005; Albrecht et al., 2008; Backman et al., 2016). However, research on pharmacological activity and qualities of glucuronides is still scarce, and their potential to act as DDI perpetrators has not been routinely screened during drug development. Moreover, clinical data demonstrating the role of CYP2C8 in the metabolism of glucuronide compounds is lacking.

The primary objectives of the present clinical study were to examine the role of CYP2C8 in desloratadine metabolism and evaluate the applicability of desloratadine as a CYP2C8 probe substrate. Moreover, we aimed to compare head-to-head the CYP2C8 inhibitory effects of the typically clinically used doses of clopidogrel and gemfibrozil in vivo in humans.

Materials and Methods

Subjects and Study Design. Twelve subjects were recruited for the study, but one of them withdrew from the study after the second phase for personal reasons. Eleven healthy nonsmoking volunteers (six women, five men; age range, 20–29 years; body mass index range, 18.6–27.3 kg/m²) participated in the study after giving written informed consent. Their health was confirmed by medical history, clinical examination, and routine laboratory tests before entering the study. All participants had normal blood platelet counts and hemoglobin values. None of the subjects used oral contraceptives or other continuous medication.

The study protocol was approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District (record number 296/13/03/00/2015), and the Finnish Medicines Agency Fimea (EudraCT number 2015-000368-32). In a randomized, placebo-controlled, three-phase crossover study, the subjects ingested the compounds in the metabolism of glucuronides is lacking.

Fig. 1. The proposed biotransformation pathways and the chemical structures of desloratadine and its 5-hydroxy, 6-hydroxy, N-glucuronide, 3-hydroxy, and 3-hydroxy-O-glucuronide metabolites (Kazmi et al., 2015a).

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phase A) and acetanilite (mobile phase B), and a solvent gradient was adopted as follows for the total run time of 11 minutes: 3 minutes at 20% B; a linear increase from 20% B to 63% B over 3 minutes; 1 minute at 90% B; 4 minutes equilibration at 20% B. The flow rate and the column temperature were maintained at 300 μl/min and 30°C, and an aliquot of 2 μl was injected into the system. A Sciex 5500 Qtrap tandem mass spectrometer (Sciex, Toronto, Ontario, Canada) interfaced with an electrospray ion source was used for the mass spectrometric detection. The mass spectrometer was operated in the positive-polarity multiple-reaction mode and the Q1 and Q3 quadrupoles were set at unit mass resolution. The mass transitions (m/z) 311 to 259, 327 to 275, and 503 to 327 were selected for desloratadine, 3-hydroxydesloratadine, and clopidogrel desbenzoate, respectively. The lower limit of quantification (LLQ) for desloratadine and 3-hydroxydesloratadine was 0.05 and 0.025 ng/ml, respectively. A signal-to-noise ratio of 3:1 was used as a limit of detection for 3-hydroxydesloratadine N-glucuronide, and the quantities were given in arbitrary units (units per milliliter) relative to the ratio of the peak heights of the 3-hydroxydesloratadine N-glucuronide to the internal standard (3-hydroxydesloratadine-d4). The day-to-day coefficients of variation (CV) were 6.0% (0.2 ng/ml), 2.6% (2.0 ng/ml), and 4.6% (20 ng/ml) for desloratadine, and 4.2% (0.1 ng/ml), 2.1% (1.0 ng/ml), and 3.5% (10 ng/ml) for 3-hydroxydesloratadine.

**Determination of Plasma Gemfibrozil and Gemfibrozil-1-O-Glucuronide.**

The plasma gemfibrozil and gemfibrozil-1-O-glucuronide were purified using a simple protein precipitation with acetonitrile (1:3) containing the corresponding deuterium-labeled internal standards, gemfibrozil-d6, and gemfibrozil-1-O-glucuronide-d6 (0.5 μM/gl). The plasma extract was then further diluted with water and introduced to the Sciex 5500 QTRAP LC-MS/MS system. The chromatographic separation of the analytes was carried out on a Kinetex C18 column (75 × 2.1-mm internal diameter, 2.6-μm particle size; Phenomenex) using 2 mM ammonium acetate (pH 4) and acetonitrile (mobile phase B). The maximum inactivation rate, \( k_{deg} \), is the rate constant of hepatic P450 degradation in the absence of the inhibitor, \( K_{m} \) is the maximum inactivation rate, \( I \) is the unbound inhibitor concentration at the enzyme site, and \( K_{i} \) is the inhibitor concentration needed to cause half of \( k_{m} \). A half-life of 22 hours for CYP2C8 was used, corresponding to \( k_{deg} \) of 0.000625 (Backman et al., 2009). The \( k_{deg} \) values were 2.82 1/min and 0.21 1/min, and \( K_{i} \) values were 9.9 μM and 20 μM for clopidogrel acetyl-β-D-glucuronide and gemfibrozil 1-O-glucuronide, respectively (Ogilvie et al., 2006; Tornio et al., 2014).

**Statistical Analysis.**

On the basis of our previous drug interaction studies, twelve subjects were estimated to be adequate to detect a 30% change in the AUC of the victim drug and its main metabolite between the placebo (control) and clopidogrel and gemfibrozil phases, with a power of at least 80% (α level 5%). The results are expressed as geometric means and geometric mean ratios with geometric CV or 90% confidence intervals (CIs) unless stated otherwise. Logarithmic transformation was used for pharmacokinetic variables, except \( t_{1/2} \) before statistical analysis. The pharmacokinetic variables were compared by repeated-measures analysis of variance with treatment phase as a within-subjects factor, followed by pairwise comparisons with Bonferroni-corrected analysis of variance. The \( t_{1/2} \) data were compared using the Wilcoxon signed rank test. Correlations between the fold-change in desloratadine AUC and the changes to gemfibrozil and gemfibrozil phases were quantified as Pearson’s correlation coefficients. P-values below 0.05 were considered statistically significant. Statistical analyses were performed using SPSS Statistics for Windows version 22.0 (IBM Corporation, Armonk, NY).

**Results**

**Effect of Clopidogrel on Parent Desloratadine.**

Compared with placebo (control), clopidogrel increased desloratadine AUC_{0–∞} to 280% (\( P = 3 \times 10^{-7} \); 90% CI 232%–338%). In addition, the \( C_{\text{max}} \) of desloratadine was increased to 165% (\( P = 0.0006; 90\% \text{ CI 133%–204%} \)) and its \( t_{1/2} \) prolonged from 17 to 26 hours (ratio to control 150%; \( P = 0.0003; 90\% \text{ CI 130%–180%} \)) by clopidogrel (Fig. 2; Table 1).

**Effect of Clopidogrel on 3-Hydroxydesloratadine.**

Clopidogrel decreased the 3-hydroxydesloratadine AUC_{0–∞} to 74% (\( P = 5 \times 10^{-5} \); 90% CI 69%–80% of control). Moreover, in the clopidogrel phase, the 3-hydroxydesloratadine:desloratadine \( AUC_{0–∞} \) ratio between clopidogrel and gemfibrozil phases were quantified as Pearson’s correlation coefficients. P-values below 0.05 were considered statistically significant. Statistical analyses were performed using SPSS Statistics for Windows version 22.0 (IBM Corporation, Armonk, NY).

**Effect of Clopidogrel on Parent Desloratadine.**

Compared with placebo (control), gemfibrozil increased desloratadine AUC_{0–∞} to 462% (\( P = 4 \times 10^{-7} \); 90% CI 346%–616%). The \( C_{\text{max}} \) of desloratadine was increased to 174% (\( P = 0.0006; 90\% \text{ CI 137%–221%} \)) and its \( t_{1/2} \) prolonged from 17 to 39 hours (ratio to control 220%; \( P = 3 \times 10^{-8} \); 90% CI 200%–250%) by gemfibrozil (Fig. 2; Table 1).
Fig. 2. The effect of clopidogrel (300 mg on day 3, followed by 75 mg on days 4 and 5) and gemfibrozil (600 mg twice daily on days 1–5) on the plasma concentrations of desloratadine (A), and its 3-hydroxy (B), and 3-hydroxy-O-glucuronide (C) metabolites. On day 3, desloratadine 5 mg was administered to 11 healthy volunteers 1 hour after the morning dose of each pretreatment. Data are presented as geometric means with 90% confidence intervals. For clarity, some error bars have been omitted. Insets depict the same data on semilogarithmic scale.
Effect of Gemfibrozil on 3-Hydroxydesloratadine. During the gemfibrozil phase, the concentrations of 3-hydroxydesloratadine were very low, and the 3-hydroxydesloratadine AUC_{0-71 h} and 3-hydroxydesloratadine:desloratadine AUC_{0-71 h} ratios were 6% (P = 2 x 10^{-8}; 90% CI 4%–9%) and 1.7% (P = 8 x 10^{-11}; 90% CI 1.3%–2.4%) of control, respectively. Gemfibrozil decreased the C_{max} of 3-hydroxydesloratadine to 4% of control (P = 8 x 10^{-10}; 90% CI 3%–5%). Owing to the extremely low 3-hydroxydesloratadine concentrations, its t_{1/2} could not be reliably determined in most of the subjects in the gemfibrozil phase.

Effect of Gemfibrozil on 3-Hydroxydesloratadine-O-Glucuronide. In the gemfibrozil phase, the AUC_{0-71 h} of 3-hydroxydesloratadine-O-glucuronide was 6% (P = 7 x 10^{-8}; 90% CI 4%–10%) and its C_{max} was 4% (P = 3 x 10^{-8}; 90% CI 2%–6%) of that in the placebo phase. In addition, the t_{max} of 3-hydroxydesloratadine-O-glucuronide was prolonged from 9.0 to 11.0 hours (P = 0.03). As with 3-hydroxydesloratadine, the plasma concentrations of 3-hydroxydesloratadine-O-glucuronide were too low for accurate calculation of its t_{1/2} during the gemfibrozil phase.

Head-to-Head Comparison of the Effects of Clopidogrel and Gemfibrozil. In general, gemfibrozil’s effect on desloratadine pharmacokinetics was stronger than clopidogrel’s (Fig. 2). By gemfibrozil, desloratadine AUC_{0-\infty} was 165% (P = 2 x 10^{-5}; 90% CI 142%–191%) of that by clopidogrel. Moreover, 3-hydroxydesloratadine AUC_{0-71 h} was 12% (P = 1 x 10^{-5}; 90% CI 9%–16%) and the 3-hydroxydesloratadine:desloratadine AUC_{0-71 h} ratio was 8% (P = 7 x 10^{-10}; 90% CI 7%–11%), compared with those in the clopidogrel phase. Additionally, in the gemfibrozil phase, the AUC_{0-\infty} of 3-hydroxydesloratadine-O-glucuronide was 11% (P = 1 x 10^{-8}; 90% CI 9%–15%) of that in the clopidogrel phase. There was a significant (Pearson two-tailed, P = 0.002; R^2 = 0.80) correlation between the fold-change in desloratadine AUC_{0-\infty} caused by clopidogrel and that by gemfibrozil (Fig. 2C).

Pharmacokinetic Variables of Clopidogrel and Gemfibrozil. The C_{max} of clopidogrel and gemfibrozil, as well as their metabolites, were reached 1–3 hours after their ingestion (Table 2), i.e., around or soon after the time of desloratadine administration. There were 50- and 20-fold interindividual variations in parent clopidogrel C_{max} and AUC_{0-\infty}, respectively. In addition, a 3- to 4-fold variation was observed in those of the active cis-5-thiol, carboxylic acid, and acyl-β-D-glucuronide metabolites of gemfibrozil. The variation in the pharmacokinetic variables of gemfibrozil and its 1-O-glucuronide metabolite was smaller, only up to 2-fold. Consistent with previous DDI studies between clopidogrel or gemfibrozil and CYP2C8 substrates (Backman et al., 2002; Jaakkola et al., 2005; Tornio et al., 2014, 2018, 2019), no significant correlations were observed between the plasma concentrations of clopidogrel acyl-β-D-glucuronide or gemfibrozil 1-O-glucuronide and changes in desloratadine pharmacokinetics.

Discussion

Recently, CYP2C8 was suggested to have an essential role in the biotransformation of desloratadine to its major metabolite, 3-hydroxydesloratadine (Kazmi et al., 2015a). Clopidogrel and gemfibrozil are both clinically relevant CYP2C8 inhibitors, but previously their CYP2C8 inhibition potency had not been compared with each other in humans. In addition, selective, sensitive, and safe CYP2C8 probe substrates are required for DDI studies. For example, repaglinide, the CYP2C8 probe drug recommended by FDA (https://www.fda.gov/downloads/drugs/guidances/ucm292362.pdf), has also other disposition mechanisms, e.g., organic anion-transporting polypeptide 1B1 (OATP1B1)-mediated active hepatic uptake and metabolism by CYP3A4 (Tornio et al., 2012), which can complicate data interpretation. For these reasons, we conducted a clinical study in healthy volunteers to investigate the role of CYP2C8 in desloratadine metabolism, and to compare the CYP2C8 inhibitory properties of clopidogrel and gemfibrozil. Overall, both clopidogrel and gemfibrozil increased the exposure to desloratadine and radically reduced the concentrations of 3-hydroxydesloratadine.
On the basis of in vitro results, Kazmi et al. (2015a) suggested an unusual metabolic pathway, by which three distinct consecutive metabolic steps are required for the formation of 3-hydroxylsloratadine (Fig. 1). First, uridine 5'-diphospho-glucuronosyltransferase (UGT) 2B10 metabolizes desloratadine to its N-glucuronide. Subsequently, the N-glucuronide undergoes rapid oxidation to 3-hydroxydesloratadine-N-glucuronide very selectively by CYP2C8 (Kazmi et al., 2015a). Finally, the glucuronide moiety is deconjugated, most probably spontaneously, during or very selectively by CYP2C8 (Kazmi et al., 2015a). Finally, the glucuronide hydroxydesloratadine and its O-glucuronide did not significantly increase desloratadine exposure, suggesting lack of these enzymes does not lead to clinically relevant changes in desloratadine pharmacokinetics in vivo (Henz, 2001; McClellan and Jarvis, 2001). Furthermore, the CYP3A4 and OATP1B1 inhibitor erythromycin was undetectable in the experiments, desloratadine was not 3-hydroxylated by CYP2C8 unless coincubated with UGT2B10, and thus the proposed 3-hydroxy metabolite is highly dependent on CYP2C8 activity in humans (Honkalammi et al., 2012).

The investigators were unable to detect desloratadine-desloratadine by UGT1A1, UGT1A3, and UGT2B15 (Ghosal et al., 2004). The resulting 3-hydroxydesloratadine is further conjugated to O-glucuronide by UGT1A1, UGT1A3, and UGT2B15 (Ghosal et al., 2004). The investigators were unable to detect desloratadine-N-glucuronide, most probably owing to its instability and rapid CYP2C8-mediated oxidation (Kazmi et al., 2015a). Although desloratadine-N-glucuronide was undetectable in the experiments, desloratadine was not 3-hydroxylated by CYP2C8 unless coincubated with UGT2B10, and thus the proposed pathway seems convincing (Kazmi et al., 2015a). Of note, desloratadine is also biotransformed to 5-hydroxy and 6-hydroxy metabolites by several P450 enzymes, namely CYP3A4, CYP2D6, and CYP2C19, in vitro (Barecki et al., 2001; McClellan and Jarvis, 2001; Kazmi et al., 2015b). However, these are only minor elimination pathways, and inhibition of these enzymes does not lead to clinically relevant changes in desloratadine pharmacokinetics in vivo (Henz, 2001; McClellan and Jarvis, 2001). Furthermore, the CYP3A4 and OATP1B1 inhibitor erythromycin did not significantly increase desloratadine exposure, suggesting lack of OATP1B1 contribution in desloratadine disposition (Henz, 2001).

In the clodigrol phase of the present study, the AUCs of 3-hydroxydesloratadine and its O-glucuronide were markedly decreased, whereas the AUC of parent desloratadine was more than doubled. The resulting reduction in 3-hydroxydesloratadine:desloratadine AUC0–71 h ratio by clodigrol is consistent with an average 80% inhibition of CYP2C8 activity, assuming that clodigrol had no effect on non-CYP2C8-mediated metabolism of desloratadine N-glucuronide nor on hydrolysis of either of the N-glucuronide intermediates. With respect to clodigrol’s CYP2C8 inhibition potency, this estimate is in accordance with recent findings that clodigrol causes about 5-fold increases in exposures to repaglinide and dasabuvir, which are among the most sensitive CYP2C8 substrates identified so far (Tornio et al., 2014; Itkonen et al., 2019).

In most individuals, gemfibrozil almost abolished 3-hydroxydesloratadine and its O-glucuronide from plasma, while reducing the 3-hydroxydesloratadine:desloratadine AUC0–71 h ratio to less than 2% of that during the placebo phase (Fig. 3A, Table 1). In addition, gemfibrozil increased desloratadine exposure in all subjects. Concerning the CYP2C8-inhibitory strength of gemfibrozil, the deductions inferred from the current results are highly concordant with the estimates from previous studies with gemfibrozil and the CYP2C8 substrate repaglinide, which indicated that clinical doses of gemfibrozil (600 mg BID) decrease CYP2C8 activity by >98% (Honkalammi et al., 2012).

The effects of gemfibrozil on desloratadine pharmacokinetics resembled those of clodigrol but were significantly more prominent, which is in line with the static predictions made on the basis of the unbound plasma Cmax of gemfibrozil and clodigrol glucuronides, and their in vitro mechanism-based inhibition parameters reported in the literature (Fig. 3F). The AUC of desloratadine was augmented 2.8-fold (range 1.8–4.7-fold) and 4.6-fold (range 2.3–7.9-fold) by clodigrol and gemfibrozil, respectively. The increases in desloratadine AUC caused by clodigrol and gemfibrozil correlated with each other (Fig. 3C), and with the 3-hydroxydesloratadine:desloratadine AUC0–71 h ratio in the placebo phase that probably reflects CYP2C8 activity at baseline (Fig. 3, D and E). These findings suggest that the basis of the effects of clodigrol and gemfibrozil is a shared CYP2C8-selective mechanism and that interindividual variation in CYP2C8 enzyme activity largely explains the variability in sensitivity of desloratadine to CYP2C8 inhibition. The present and previous reports imply that clinical doses of gemfibrozil cause stronger interactions with CYP2C8 substrates than those of clodigrol, thus revealing more sensitivity with CYP2C8 participates in the metabolism of the victim drug (Table 3). However, gemfibrozil also inhibits the activity of certain membrane transporters, most notably hepatic OATP1B1 (Neuvonen et al., 2006; Tornio et al., 2017), which in turn is not inhibited by clodigrol to a clinically relevant extent (Itkonen et al., 2015; Kim et al., 2016). Therefore, interpreting the DDIs mechanisms can be challenging when gemfibrozil 600 mg BID is used as a model CYP2C8 inhibitor, if the victim drug is also a substrate of OATP1B1, as repaglinide and cerivastatin are (Backman et al., 2002, 2016; Niemi et al., 2003; Shiota et al., 2003; Niemi et al., 2005; Ogilvie et al., 2006; Tornio et al., 2017). In such cases, clodigrol could be considered as an alternative model inhibitor.

Our current results imply that the metabolism of desloratadine to its 3-hydroxy metabolite is highly dependent on CYP2C8 activity in humans in vivo. Although gemfibrozil and clodigrol increased the AUC of desloratadine less than they had increased the AUCs of repaglinide and dasabuvir (Table 3), the 3-hydroxydesloratadine:desloratadine ratio seemed to be highly sensitive to CYP2C8 inhibition. These observations corroborate the previous in vitro findings of Kazmi et al. (2015a) and suggest the possibility of using desloratadine as a CYP2C8 probe substrate. However, an important caveat is that the formation of 3-hydroxydesloratadine is also dependent on UGT2B10 activity (Kazmi et al., 2015b) and can probably be altered by inhibition and induction of UGT2B10. Of note, neither clodigrol nor gemfibrozil is
Fig. 3. The individual change in 3-hydroxydesloratadine:desloratadine area under the plasma concentration time curve (AUC0–71 h) ratio (A) and desloratadine AUC∞ (B) in the clopidogrel and gemfibrozil phase in comparison with placebo phase value. The correlation of desloratadine AUC∞ ratio between gemfibrozil to control and clopidogrel to control (C), and the correlation of 3-hydroxydesloratadine:desloratadine AUC0–71 h ratio in the placebo phase with desloratadine AUC0–∞ clopidogrel to control ratio (D), and desloratadine AUC0–∞ gemfibrozil to control ratio (E), where the lines depict the linear regression with 95% CI. Predicted CYP2C8 inhibitory effect of gemfibrozil and clopidogrel using a static prediction equation whose basis was in vitro mechanism-based inhibition parameters of their glucuronide metabolites reported in the literature and their concentrations at the enzyme site (F). See Materials and Methods for details of the prediction equation.

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Cmax,u,clop, unbound peak plasma concentration of clopidogrel acyl-D-glucuronide after 300 mg clopidogrel in the current study (0.83 μM with an estimated fraction unbound of 10%); Cmax,u,gemfi, unbound peak plasma concentration of gemfibrozil 1-O-glucuronide after 600 mg gemfibrozil in the current study (3.9 μM with an estimated fraction unbound of 11.5%).
known to inhibit UGT2B10. Moreover, in some individuals, the \( t_{1/2} \) and drug exposure of desloratadine are very high, resulting in a “poor metabolizer” phenotype, which has a population frequency of 17% in African-Americans and 8% in Native American but is less common in Caucasians and Hispanics, with a frequency of about 2% (Molimard et al., 2004; Prenger et al., 2006; Ramanathan et al., 2007). The “poor metabolizer” phenotype and almost complete CYP2C8 inactivation by gemfibrozil result in surprisingly similar exposures to desloratadine and its 3-hydroxy metabolite (Prenner et al., 2006). However, the most probable explanation for this phenotype is that common UGT2B10 variants prevent the expression of functional UGT2B10 (Berg et al., 2010a, 2010b; Chen et al., 2010; Murphy et al., 2014; Fowler et al., 2015). Another possibility for this phenomenon is variability in CYP2C8, even though nonfunctional alleles in CYP2C8 are rare in all major populations (Zhou et al., 2017). In any case, individuals with desloratadine “poor metabolizer” phenotype are probably less sensitive to changes in desloratadine pharmacokinetics by CYP2C8 inhibitors. Consequently, desloratadine might be suboptimal as a CYP2C8 probe substrate in, for example, African-Americans. From another perspective, the good tolerability of desloratadine and its 3-hydroxy metabolite (Prenner et al., 2006). However, the most probable explanation for the effects of the CYP2C8 inhibitors gemfibrozil and clopidogrel on the pharmacokinetics of desloratadine is inhibition of CYP2C8-mediated 3-hydroxylation of a glucuronide metabolite of desloratadine, our results provide clinical evidence supporting the findings of several in vitro studies that CYP2C8 has the ability to oxidize glucuronide metabolites in humans (Backman et al., 2016). On the basis of X-ray crystallographic studies, the large CYP2C8 active site cavity is capable of binding substrates diverse in structure (Schoch et al., 2008). As demonstrated in molecular docking simulations, there is a hydrophilic region in the active site that can accommodate the glucuronide moiety of certain metabolites, including clopidogrel acyl-\( \beta \)-D-glucuronide and gemfibrozil 1-O-glucuronide (Baer et al., 2009; Tornio et al., 2014; Backman et al., 2016), in contrast to most other P450 enzymes. Accordingly, molecular modeling combined with in vitro experiments provide useful means to predict CYP2C8-involved DDI potential of drugs and their metabolites.

As the most probable explanation for the effects of the CYP2C8 inhibitors gemfibrozil and clopidogrel on the pharmacokinetics of desloratadine is inhibition of CYP2C8-mediated 3-hydroxylation of a glucuronide metabolite of desloratadine, our results provide clinical evidence supporting the findings of several in vitro studies that CYP2C8 has the ability to oxidize glucuronide metabolites in humans (Backman et al., 2016). On the basis of X-ray crystallographic studies, the large CYP2C8 active site cavity is capable of binding substrates diverse in structure (Schoch et al., 2008). As demonstrated in molecular docking simulations, there is a hydrophilic region in the active site that can accommodate the glucuronide moiety of certain metabolites, including clopidogrel acyl-\( \beta \)-D-glucuronide and gemfibrozil 1-O-glucuronide (Baer et al., 2009; Tornio et al., 2014; Backman et al., 2016), in contrast to most other P450 enzymes. Accordingly, molecular modeling combined with in vitro experiments provide useful means to predict CYP2C8-involved DDI potential of drugs and their metabolites.

In conclusion, our clinical study revealed two DDIs with interesting characteristics and implications. First, in the clopidogrel-desloratadine and gemfibrozil-desloratadine interactions, both the perpetrator and victim compounds are glucuronide metabolites, highlighting the need to consider the properties of xenobiotic glucuronides with respect to evaluation of the risk of pharmacokinetic DDIs. Second, despite its complex metabolism, desloratadine could serve as a safe CYP2C8 probe substrate in clinical DDI studies owing to the CYP2C8-dependent 3-hydroxydesloratadine formation. It could be especially useful if the perpetrator also inhibits CYP3A4 or OATP1B1, because it differs from other recommended CYP2C8 probe substrates that are also dependent on CYP3A4 and OATP1B1. However, the suggested sequential metabolism and obligatory role of UGT2B10 prior to CYP2C8-mediated oxidation of desloratadine (Kazmi et al., 2015a) may complicate its DDI interpretation. Therefore, the applicability of desloratadine in populations with a high prevalence

| Victim Drug and Its Dose | Clopidogrel 300 mg followed by 75 mg QD Ratio (R) to Placebo (90% or 95% CI or range) | Gemfibrozil 600 mg BID Ratio (R) to Placebo (90% or 95% CI, or Range) |
|--------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| **AUC**<sub>0</sub>–<sub>24</sub> | **C**<sub>max</sub> | Reference | **AUC**<sub>0</sub>–<sub>24</sub> | **C**<sub>max</sub> | Reference |
| **Dasabuvir** | 250 mg | 4.7 (90% CI = 3.2–6.7) | 1.6 (90% CI = 1.0–2.6) | Itkonen et al. (2019) | 11.3 (90% CI = 9.1–14) | 2.0 (90% CI = 1.7–2.4) | Menon et al. (2015) |
| **Desloratadine** | 5 mg | 2.8 (90% CI = 2.3–3.4) | 1.7 (90% CI = 1.3–2.0) | Present study | 4.6 (90% CI = 3.5–6.2) | 1.7 (90% CI = 1.4–2.2) | Present study |
| **Montelukast** | 10 mg | 2.0 (90% CI = 1.7–2.3) | 1.0 (90% CI = 0.8–1.2) | Itkonen et al. (2018) | 4.4 (95% CI = 3.8–5.0) | 1.5 (95% CI = 1.3–1.8) | Karonen et al. (2010) |
| **Pioglitazone** | 1 mg | 2.0 (95% CI = 1.6–2.5) | Not reported | Kim et al. (2016) | 3.2 (Range = 2.3–6.5) | 1.1 (Range = 0.5–2.6) | Juakkola et al. (2005) |
| **15 mg** | 2.1 (90% CI = 1.8–2.6) | 1.0 (90% CI = 0.8–1.4) | Itkonen et al. (2016) | 3.4 (Range = 2.3–6.2) | Not reported | Deng et al. (2005) |
| **30 mg** | Repaglinide | 0.1 mg | 3.1 (95% CI = 2.1–4.1) | Not reported | Kim et al. (2016) | 8.1 (Range = 5.5–15) | 2.4 (Range = 1.7–6.1) | Niemi et al. (2003) |
| **0.25 mg** | 5.1 (90% CI = 3.9–6.6) | 2.5 (90% CI = 1.8–3.5) | Tornio et al. (2014) | 7.0 (Range = 2.9–14) | 2.2 (Range = 1.4–2.9) | Tornio et al. (2008) |
| | 3.9 (90% CI = 2.9–5.3) | 2.0 (90% CI = 1.3–3.1) | Tornio et al. (2014) | 7.6 (Range = 4.2–12) | 2.7 (Range = 1.5–3.7) | Backman et al. (2009) |
| | | | | 7.0 (90% CI = 6.0–8.1) | 2.0 (90% CI = 1.6–2.5) | Honkalammi et al. (2012) |

**AUC**<sub>0</sub>–<sub>24</sub>, area under the plasma concentration–time curve from 0 to 8 hours; **AUC**<sub>0</sub>–<sub>24</sub>, area under the plasma concentration–time curve from 0 to 24 hours; **AUC**<sub>max</sub>, area under the plasma concentration–time curve from time 0 to infinity.

**AUC**<sub>0</sub>–<sub>24</sub>: ratio.

**AUC**<sub>0</sub>–<sub>24</sub>: ratio.

**Effect on day 3 of treatment with clopidogrel 300 mg on day 1 followed by 75 mg QD.**
