Glucuronidation of DRF-6574, hydroxy metabolite of DRF-4367 (a novel COX-2 inhibitor) by pooled human liver, intestinal microsomes and recombinant human UDP-glucuronosyltransferases (UGT): Role of UGT1A1, 1A3 and 1A8*

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SUMMARY

DRF-4367 is a novel COX-2 inhibitor, which showed good efficacy in several animal models of inflammation. In a comparative in vitro metabolism in various liver microsomes, DRF-4367 forms a hydroxy metabolite (DRF-6574) mediated by CYP2D6 and 2C19 isoenzymes. DRF-6574 readily undergoes Phase-II metabolism and forms glucuronide and sulfate conjugates both in vitro and in vivo. The objective of the present study was two folds: to study the glucuronidation of DRF-6574 in human liver and intestinal microsomes and to identify the recombinant human liver and intestinal UDP-glucuronosyltransferase (UGT) enzymes responsible for glucuronidation of DRF-6574. Of twelve recombinant UGTs tested, two hepatic UGTs viz., UGT1A1 and 1A3 and an extra hepatic UGT i.e., UGT1A8 showed the catalytic activity. The enzyme kinetics in pooled human liver, intestinal and recombinant UGT microsomes showed a typical Michaelis-Menten plot. The apparent K<sub>m</sub> and V<sub>max</sub> value for DRF-6574 was found to be 116 ± 24 μM and 2.07 ± 0.12 μg/min/mg protein and 142 ± 17 μM and 3.83 ± 0.15 μg/min/mg protein in pooled human liver and intestinal microsomes, respectively. The intrinsic clearance (V<sub>max</sub>/K<sub>m</sub>) value for DRF-6574 was estimated to be 0.043 and 0.065 ml/min/mg protein, respectively in pooled human liver and intestinal microsomes. Moreover we have determined the K<sub>m</sub> and V<sub>max</sub> and intrinsic clearance values for specific UGTs viz., UGT 1A1, 1A3 and 1A8. The apparent K<sub>m</sub> and V<sub>max</sub> values are 23 ± 7.2 μM, 3.44 ± 0.17 μg/min/mg protein for UGT1A1, 60 ± 7.9 μM, 3.67 ± 0.11 μg/min/mg protein for UGT1A3, 96 ± 8.0 μM, 2.95 ± 0.06 μg/min/mg protein for UGT1A8. The intrinsic clearance values (V<sub>max</sub>/K<sub>m</sub>) estimated were 0.367, 0.148, 0.074 ml/min/mg protein for UGT1A1, 1A3 and 1A8, respectively. The intrinsic clearance value in UGT1A8 was very close to that in human intestinal and liver microsomes. The formation of DRF-6574 glucuronide by human liver, intestinal and UGT1A1, 1A3 and 1A8 microsomes was effectively inhibited by phenylbutazone.
### INTRODUCTION

UDP glucuronosyltransferases (UGTs) are a superfamily of membrane bound enzymes located in the endoplasmic reticulum that catalyze the conjugation of endogenous substances (e.g. bilirubin, steroids, thyroid hormone) and xenobiotics with D-glucuronic acid [1]. Many different genes and pseudogenes have been identified in the UGT superfamily and are subdivided into the UGT1A and 2B families based on the sequence identity [2]. To date seventeen UGT isoforms have been identified viz., 1A1, 1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A9, 1A10, 2A1, 2B4, 2B7, 2B10, 2B11, 2B15, 2B17 and 2B18 [3]. The majority of these isoforms are expressed in liver, although UGT2A1 is mainly localized in nasal epithelium and UGT1A7, 1A8 and 1A10 apparently occur only in the gastrointestinal tract. Although liver appears to be the major organ involved in glucuronidation, some UGT isoforms exist at high levels in the kidney and intestine suggesting extra hepatic glucuronidation can be significant [4,5]. Polymorphism in UGT 1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7 and 2B15 have been identified in different populations [6-18]. Sometimes, these polymorphism results in altered enzyme expression or activities towards a variety of endogenous and xenobiotic aglycones [6,11,13,15,16,18]. UGTs catalyze the conversion of lipophilic molecules into more polar hydrophilic glucuronides, thereby facilitating their elimination via bile, feces, and urine. Fischer et al. [19] described the role of hepatic and extrahepatic UGTs and their expression in gastrointestinal tract. UGT catalyzed glucuronidation reactions are responsible for ~35% of the drugs metabolized by Phase-II enzymes [20]. There appears to be sex [21,22] and developmental differences [22,23] in the expression of specific UGT enzymes. Available evidence suggests that the individual UGTs exhibit distinct, but overlapping substrate and inhibitor selectivity and differ in terms of regulations [3].

Glucuronidation has become increasingly important in pharmaceutical drug development because biotransformation and elimination of drugs by this pathway may influence their potency, bioavailability and pharmacokinetics. The determination of the identification of the enzymes involved in drug metabolism is important to understand variation in drug effect and metabolism. Moreover since many drug metabolizing enzymes (CYPs and UGTs) are involved in the metabolism of several xenobiotics, the determination of the enzyme involved in the metabolism of a specific drug could provide essential information about potential drug interactions [24,25].

Cyclooxygenase (COX) is the key enzyme in the biosynthesis of prostanoids, biologically active substances that are involved not only in several physiological processes but also in pathological conditions such as inflammation. COX catalyzes the conversion of arachidonic acid into prostaglandin and thromboxane was considered for a long time to be responsible for both the therapeutic and adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs) [26-28]. COX enzyme exists in two forms: COX-1 and COX-2. Both enzymes are sensitive to inhibition by conventional NSAIDs. COX-1 is the constitutive isoform and is mainly responsible for the synthesis of cytoprotective prostaglandin in the gastrointestinal tract and of the pro-aggregatory thromboxane in blood platelets. COX-2 is inducible form and short lived; its expression is stimulated in response to endotoxin, cytokines and mitogens. COX-2 expression is associated with inflammation and other pathologies such as cancer proliferation, have led to the development of COX-2 selective inhibitors to improve therapeutic potency and to reduce the classical side effects associated with the use of conventional NSAIDs. Celecoxb is the first specific inhibitor of COX-2 approved by US FDA for the treatment of patients with rheumatoid arthritis, osteoarthritis and pain management [29]. Recently, a few diarylheterecycle coxibs, namely valdecoxib and etoricoxib have been commercialized for the treatment of certain inflammatory conditions. In the course of a program aiming to discover new molecules, our efforts in synthesis and systematic structural activity relationship studies identified DRF-4367 as a potent and novel diaryl pyrazole COX-2 inhibitor. DRF-4367 (Fig. 1) is chemically 2-hydroxymethyl-4-[5-(4-methoxyphenyl)-3-trifluoromethyl-1H-1-pyrazolyl]-1-benzene sulfonamide. It exhibited potent COX-2 selectivity in the in vitro studies and showed good efficacy in several animal models of inflammation [30,31]. DRF-4367 has exhibited good dose proportional pharmacokinetics with an absolute oral bioavailability of ~70-80% in Wistar rats [32]. Of late, Bhambidipati et al. [33] have reported the absolute oral bioavailability and metabolism in mice and comparative inter-species in vitro metabolism for DRF-4367. In liver microsomes of various animal species, the primary route of metabolism for DRF-4367 was demethylation of benzylmethoxy to form a hydroxy metabolite (DRF-6574). The formation of DRF-6574 was mediated by CYP2D6 and 2C19 isoenzymes [33]. O-glucuronide and O-sulfate conjugates of DRF-6574 were observed when DRF-4367 was incubated in liver microsomes of mice, rat, dog, monkey and human liver microsomes as well as in bile and urine of mice [33].

The objective of this study was to identify the human UGTs responsible for the formation of O-glucuronide of DRF-6574. In vitro incubations with cDNA expressed recombinant human UGTs and pooled human liver and intestinal microsomes were performed to determine