Accelerated Communication

In Vitro Metabolic Activation of Lumiracoxib in Rat and Human Liver Preparations

Ying Li, J. Greg Slatter, Zhoupeng Zhang, Yan Li, George A. Doss, Matthew P. Braun, Ralph A. Stearns, Dennis C. Dean, Thomas A. Baillie, and Wei Tang


Received September 24, 2007; accepted November 7, 2007

ABSTRACT:

Recent clinical reports have suggested that the cyclooxygenase-2 inhibitor, lumiracoxib (Prexige), may cause a rare but serious hepatotoxicity in patients. In view of the close structural resemblance between lumiracoxib and diclofenac, a widely used nonsteroidal anti-inflammatory drug whose use also has been associated with rare cases of liver injury, it is possible that the toxicity of the two agents may share a common mechanism. Because it is believed that chemically reactive metabolites may play a role as mediators of diclofenac-mediated hepatotoxicity, the present in vitro study was carried out to test the hypothesis that lumiracoxib also undergoes metabolic activation when incubated with liver microsomal preparations and hepatocytes from rats and humans. By means of liquid chromatography tandem mass spectrometry and nuclear magnetic resonance spectrometry techniques, two previously unknown N-acetylcysteine (NAC) conjugates were identified, namely, 3’-NAC-4’-hydroxy lumiracoxib (M1) and 4’-hydroxy-6’-NAC-desfluoro lumiracoxib (M2), the structures of which reveal the intermediacy of an electrophilic quinone imine species. Based on the results of studies with immunoinhibitory antibodies, it was demonstrated that the formation of M1 and M2 in human liver microsomes was catalyzed by cytochrome P450 (P450) 2C9. These findings demonstrate that lumiracoxib is subject to P450-mediated bioactivation in both rat and human liver preparations, leading to the formation of a reactive intermediate analogous to species generated during the metabolism of diclofenac.

Lumiracoxib [2-(2’-chloro-6’-fluorophenyl)-amino-5-methylbenzenacetic acid] (Prexige; Novartis, Basel, Switzerland) (Fig. 1) is a selective cyclooxygenase-2 inhibitor approved in over 50 countries worldwide for the treatment of osteoarthritis and acute pain (Bannwarth and Berenbaum, 2007; Buvanendran and Barkin, 2007). Recently, concerns have been raised over the clinical safety of lumiracoxib after reports of rare but serious liver reactions to the drug. Thus, eight cases of lumiracoxib-associated hepatotoxicity were reported in Australia, resulting in two deaths and two liver transplants. In light of these adverse reactions, the Australian Therapeutic Goods Administration cancelled the registration of the drug in that country in August, 2007, and the higher doses (200 and 400 mg) of lumiracoxib now have been removed from the New Zealand market by the Medicines and Medical Devices Safety Authority (http://www.medsafe.govt.nz/hot/media/2007/070811-lumiracoxib.htm; and http://www.medsafe.govt.nz/hot/media/2007/070811-lumiracoxib.htm; and http://www.medsafe.govt.nz/hot/media/2007/prexige.asp).

From a structural standpoint, lumiracoxib is a close relative of diclofenac, a drug itself known to induce a rare but severe hepatotoxicity in exposed patients. Although the precise mechanism of diclofenac-mediated hepatotoxicity remains elusive, it has been proposed that chemically reactive metabolites of the drug may play a causative role (Boelsterli, 2003; Tang, 2003). Thus, diclofenac is known to undergo cytochrome P450 (P450)-catalyzed hydroxylation at the 4’- and 5-positions, the products of which may be further oxidized to reactive quinone imine intermediates that have been characterized indirectly as their corresponding glutathione adducts (Tang, 2003). In the case of lumiracoxib, a methyl substituent occupies the 5-position, whereas the dichloroaniline moiety of diclofenac has been replaced by a fluorochloroaniline ring system (Fig. 1). Despite these structural changes, the potential remains for metabolic activation of lumiracoxib to a quinone imine to occur through hydroxylation at the exposed 4’-position. The present investigation was designed, therefore, to test the hypothesis that lumiracoxib undergoes P450-mediated metabolism to a reactive quinone imine intermediate when incubated with liver microsomal preparations and rat and human liver primary hepatocytes.

Abbreviations: P450, cytochrome P450; CID, collision-induced dissociation; LC-MS/MS, liquid chromatography tandem mass spectrometry; NAC, N-acetylcysteine; NMR, nuclear magnetic resonance spectrometry; M1, 3’-NAC-4’-hydroxy lumiracoxib; M2, 4’-hydroxy-6’-NAC-desfluoro lumiracoxib.
preparations from rat and human subjects and that the putative electrophilic species may be captured as a thioether adduct(s) with the model nucleophile \(N\)-acetylcysteine (NAC). The results of this preliminary study confirm that lumiracoxib does undergo such bioactivation in vitro.

**Materials and Methods**

**Materials.** Lumiracoxib was prepared according to methods described in the international patent application WO 01/23346. Rat and human liver microsomes and monoclonal inhibitory anti-CYP2C9 and anti-CYP3A4 antibodies were prepared at Merck Research Laboratories (West Point, PA) (Shou et al., 2000). Cryopreserved human hepatocytes from three donors and InVitro GRO Hepatocyte Media were obtained from In Vitro Technologies (Baltimore, MD). All other reagents were from commercial sources.

**Analytical Methods.** LC-MS/MS was carried out on a Finnigan LCQ Deca XP Plus ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) with IonSpray in the positive ion detection mode. High-performance liquid chromatography was performed on a Phenomenex (Torrance, CA) Synergi Hydro-RP column (4.6 \(\times\) 50 mm, 4 \(\mu\)m) operated at a flow rate of 1 ml/min. The mobile phase consisted of aqueous acetonitrile containing 0.05% acetic acid and 1 mM ammonium acetate and was programmed via a linear increase of acetonitrile content from 5 to 76% over a period of 30 min. \(^1\)H NMR spectra were recorded on a Varian (Palo Alto, CA) Inova 600 spectrometer operated at 600 MHz.

**In Vitro Metabolism.** Incubations of lumiracoxib (50 \(\mu\)M) with rat or human liver microsomes (1 mg protein/ml) were performed in the presence of NADPH (1 mM) and NAC (5 mM) at 37°C for 60 min. The incubation medium was phosphate buffer (100 mM, pH 7.4) containing EDTA (1 mM), and the volume was 1 ml. Incubations in immunoinhibitory studies were performed in a similar manner, except that the anti-CYP2C9 or 3A4 antibody was preincubated with human liver microsomes for 15 min at room temperature before initiation of lumiracoxib metabolism. The reaction mixture was acidified to pH 2 to 3 with 10% aqueous trifluoroacetic acid and extracted with an Oasis HLB extraction cartridge (Waters Co., Milford, MA).

Incubations of lumiracoxib (50 \(\mu\)M) with human hepatocytes (1 \(\times\) 10^6 cells/ml) were performed in the presence of NAC (5 mM) at 37°C for 60 min. The incubation medium was InVitro GRO Hepatocyte Media, and the volume was 5 ml. After acidification to pH 2 to 3 with 10% aqueous trifluoroacetic acid, the cells were lysed via sonication, and the mixture was extracted with an Oasis HLB extraction cartridge.

The resulting samples were analyzed by LC-MS/MS. Samples from liver microsomal incubations (200 ml incubation) were further purified by high-performance liquid chromatography for NMR analysis.

**Results and Discussion**

Two NAC conjugates, M1 and M2, were identified by LC-MS/MS analysis after incubations of lumiracoxib with rat and human liver.
In human liver microsomal incubations (Yu et al., 2005), resulting from thiol substitution of chlorine was detected in human liver microsomes. The quinone imine intermediate then is captured by NAC to afford the thioether conjugates \( M1 \) and \( M2 \). Formation of \( M1 \) is of mechanistic interest as it involves a selective displacement of fluorine (as opposed to chlorine) by NAC. A plausible explanation for this observation is that, compared with chlorine, the smaller size and stronger electron-withdrawing effect of fluorine renders the fluorinated carbon atom a preferred target for attack by nucleophiles such as NAC, and the overall process is dictated by the kinetics of the initial nucleophilic addition step as opposed to the subsequent halide elimination. Interestingly, in the case of diclofenac, which possesses a dichloroaniline moiety instead of a fluorochloroaniline ring system, a glutathione adduct resulting from thiol substitution of chlorine was detected in human liver microsomal incubations (Yu et al., 2005).

The identification of the NAC conjugates \( M1 \) and \( M2 \) in human hepatocyte suspensions suggests that lumiracoxib metabolism to reactive quinone imine intermediate(s) may take place in patients. In addition to forming thiold adducts, quinone imines are known to be capable of covalently modifying cellular proteins and have been...
implicated in a number of drug-related adverse effects (Guengerich and MacDonald, 2007; Tang, 2007). It should be noted that neither M1 nor M2 (nor other thioether conjugates) was detected in a single-dose study of lumiracoxib metabolism in four healthy male subjects (Mangold et al., 2004). However, in light of the recent withdrawal of lumiracoxib from the Australian market and its restricted use in New Zealand, further studies of the metabolic fate of the drug in humans may be warranted to better understand the relationship between lumiracoxib metabolism and its adverse hepatic effects.

FIG. 5. Upper panel: 1D 1H NMR (aromatic region) spectrum of M2, 4'-hydroxy-6'-NAC-desfluoro lumiracoxib. Lower panel: 2D nuclear Overhauser effect (rotating frame nuclear Overhauser enhancement spectroscopy) NMR spectrum of M2. The signals are discussed in the text. RS represents N-acetylcysteine.
Acknowledgments. We thank Dr. Deborah Nicoll-Griffith, Yuming Zhao, and colleagues in the Department of Drug Metabolism and Pharmacokinetics for valuable discussions and for providing lumiracoxib, liver microsomes, and inhibitory anti-P450 antibodies.

Fig. 6. Proposed pathways for the formation of NAC conjugates M1 and M2 as metabolites of lumiracoxib in rat and human liver preparations.

References

Address correspondence to: Dr. Wei Tang, RY800-B200, Merck Research Laboratories, Rahway, NJ 07065-0900. E-mail: wei_tang@merck.com