Deuterium-Substituted Lapatinib Isoloplogs Exhibit Reduction in O-debenzylation and Metabolic Shunting

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Abstract

Lapatinib (1) [Tarceva®] is a potent, orally available small-molecule reversible dual EGFR and HER2 tyrosine kinase inhibitor. It is indicated, in combination with capecitabine, for the treatment of advanced or metastatic breast cancer. In humans, lapatinib undergoes extensive metabolism and is eliminated primarily in the feces. The predominant metabolite observed in human feces is the putatively reversible and hence undehydrodeuterated phenol M1 (Fig 1). Previous studies from CYP3A4-mediated O-debenzylation of lapatinib showed the effects of deuteration on the metabolite fate of the aryl gly, while deuterium isotope effects on CYP3A4-mediated metabolism are well-documented. The consequences of deuterium substitution on the overall metabolite profile of a drug are unpredictable. Hence, in vivo or in vitro testing is required to determine the effects of deuteration-substitution on the metabolism and pharmacokinetics of a drug. We investigated the formation of the O-debenzylation metabolite M1 from nine deuterated lapatinib analogs in human liver microsomes (HLM). A small reduction in the formation of the phenolic metabolite compared to lapatinib. Interestingly, several of the more heavily deuterated analogs showed increased affect on the formation of the phenolic metabolite. Kinetic isotope effects, \( V' \) and \( V'' \), for a d2-isotopolog were also observed. In addition, metabolite profiling in hepatocytes was performed. The results of these experiments supported the observations from the HLM studies and are indicative of metabolic shunting.

Introduction

Lapatinib has been reported to cause diarrhea and hypertension and its clinical use has been associated with fatalities in a few cases. Lapatinib is metabolized by CYP3A4 (isozyme) and CYP2D6 (minor) to the O-dehydrodeuterated and 1H-hydroxylated metabolites in vivo (Fig 1). This profile provides evidence that the O-debenzylation or phenolic metabolites is bioactivated to a reactive intermediate and suggests that the metabolite may be responsible for the drug’s hepatotoxicity. Lapatinib has also been observed to cause mechanism-based inactivation (MBI) of CYP3A4. A recent report demonstrated that the CYP3A4 MBI by lapatinib is caused by a quasireversible MBI complex formation proposed to be mediated via Pyrroline-based of the secondary amino group followed by the formation of a reactive nitrile intermediate. The same report continues to associate MBI with the hepatotoxicity observed with lapatinib.

Deuterium-substitution has the potential to alter the metabolic fate of drugs that are biotransformed by cytochrome P450 (CYP) mediated carbon-hydrogen bond cleavage. Deuterium isotope effects may decrease the rate of formation of metabolites that result from such mechanisms due to a stronger carbon-deuterium bond. In the present study we report the effects of deuteration of lapatinib on the formation of M1. Several deuterated lapatinib isoloplogs (lapatinib) were investigated (Fig 2). Studies performed in HLM and human hepatocytes indicate that the substrates that were deuterated at the benzylic carbon form less of M1. Metabolite profiling in human hepatocytes suggest that the decrease in M1 may be accompanied by an increase in M1 as a result of metabolic shunting, a phenomenon known to be associated with deuteration-substitution.

Materials and Methods

Lapatinib and its isotopologs were synthesized at Concert Pharmaceuticals Inc. Phenolic metabolite standards (Fig 2) were synthesized at Concert. Comparison of the amount of the metabolite formed (Fig 2). Table 3 was performed in HLM (pool of 200, mixed gender, 0.5 mg/mL, final concentration) with 25 mM NaCl, 2 mM NaHPO4, 1 mM MnCl2, pH 7.4. Reaction mixtures were incubated for 10 min at 37°C and were stopped by addition of acetonitrile. Amount of metabolite formed was quantitated by LC-MS/MS. Similar incubation conditions were used for \( K_m \) and \( V_{max} \) determination (Fig 4). For semi-quantitative analyses of metabolites by (Table 3) 25 mM or d2- lapatinib was incubated with human hepatocytes (A170 liver cells) at 37°C for 4h. Quantitative analyses of metabolites by (Fig 4, Table 3) and 25 mM or d2- lapatinib was incubated with human hepatocytes (A170 liver cells) at 37°C for 4h.

Results

1. A large reduction (> 60-70% percent) in the amount of the phenolic metabolite (M1 or M11) was observed in HLM with 1a, 1b, and 1c (Table 3) of which were deuterated at the benzylic position. All additional d-isotopologs tested also produced less of the phenolic metabolite (M1 > 20-35% reduction with the exception of 1a). Compound 1a was deactivated at the site of ketonization and not at the benzylic position. Compounds 1a and 1b were also not deactivated at the benzylic position however these two isotopologs formed approximately 20-35% less of phenolic metabolite compared to lapatinib.

2. \( K_m \) and \( V_{max} \) values for the formation of M1 from lapatinib and compound 1a (d1-lapatinib) were determined in HLM (Figure 4). The \( V_{max} \) for lapatinib was more than two-fold higher than that for 1a, resulting in a large deuterium isotope effect (\( V' \)/\( V'' \) = 2.4). The \( K_m \) values for the two compounds were similar, and hence the intrinsic clearance of lapatinib was about two-fold higher than that of 1a and \( V' \)/\( V'' \) = 2. Thus deuterium substitution at the benzylic position resulted in substantial kinetic isotope effects on the formation of the M1 and is mediated primarily on the velocity (\( V' \)/\( V'' \)) and not on the apparent affinity (\( K_m \)) for the reaction.

3. Metabolites of lapatinib and compound 1a (d1-lapatinib) were profiled in human hepatocytes (Fig 5) and were found to be metabolically stable, with deuterium having little effect on the formation of metabolites. Metabolic profiling of lapatinib in human hepatocytes showed that M1, M3 and M11 were the predominant metabolites generated with both lapatinib and d2-lapatinib in human hepatocytes. (Fig 5) 1a which resulted in a decrease of hydroxylation of the quinazoline ring has been reported previously. The relative amount of M1 formed from 1a was approximately half that of the amount formed from lapatinib (Fig 5). Interestingly, the relative amount of M11 formed from 1a increased compared to the amount formed from lapatinib. These results indicate that the metabolism of lapatinib from M1 to M11 when deuterated at the benzylic position. A small reduction in the formation of M0 from 1a compared to lapatinib was also observed.

Conclusions

1. Deuteriation of lapatinib at the site of O-debenzylation as in d2-lapatinib (1a) resulted in a substantial deuterium isotope effect, \( V' \)/\( V'' \) = 2.4. This effect caused a reduction in the formation of the phenolic metabolite (M1) from lapatinib.

2. The phenolic metabolite has been shown to generate a reactive quinazoline and has been implicated in the hepatotoxicity observed with the clinical use of lapatinib. The reduction in the amount of the phenolic metabolite 1a has been associated with the clinical use of lapatinib.

Literature cited


