CYP4F2 is the Major Cytochrome P450 Enzyme Involved in CMX001 Metabolism

Tim Tippin[1], Jenni Chladek[2], Laurie Keilholz[1], Etsuko Usuki[2], Brian Ogilvie[2], Kathy Van Sickle[1], Herve Mommeja-Marin[1], Irma M. Grossi[1] and Lawrence C. Trost[1]

[1]Chimerix, Inc; [2]XenoTech, LLC

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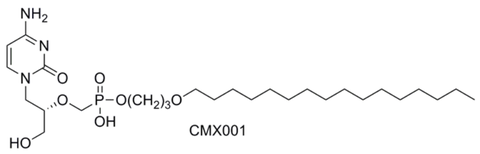
Abstract

CMX001 (brincidofovir) is a novel, broad spectrum, orally administered, lipid acyclic nucleotide phosphonate in development for prevention and/or treatment of diseases caused by double-stranded DNA viruses, including cytomegalovirus, BK virus and adenovirus. Previous in vitro studies identified CYP3A4, 2C8, 2C19 and 2E1 as the cytochrome P450 (CYP) enzymes responsible for CMX001 metabolism. The objective of this study was to quantify the percentage of CMX001 metabolized by each CYP, and to explore potential metabolic pathways for CMX001 via less traditional CYPs known to metabolize endogenous long chain fatty acids. Pooled human liver microsomes, recombinant human CYP enzymes (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2J2, 3A4, 4A11, 4F2, 4F3a, 4F3b and 4F12) and specific CYP inhibitors were used for this study.

Incubations with recombinant human CYP enzymes suggested that only CYP4F2 metabolizes CMX001. The complete inhibition of CMX001 metabolism in human liver microsomes by N-hydroxy-N'-(4-n-butyl-2-methylphenyl)formamidine (HET0016), a specific CYP4F2/3 inhibitor, and the partial inhibition by ketoconazole, a dual CYP3A and CYP4F2 inhibitor, confirmed the recombinant CYP results. The near complete inhibition of CMX001 metabolism by quercetin (CYP2C8 inhibitor), disulfiram (CYP2E1 inhibitor) and tranylcypromine (CYP2C19 inhibitor) was also observed in human liver microsomes; however, subsequent experiments performed with recombinant CYP4F2 alone indicated that these chemicals also inhibit CYP4F2-mediated metabolism of CMX001. Thus, inhibition of microsomal metabolism of CMX001 by these inhibitors is likely due to inhibition of CYP4F2 rather than inhibition of CYP2C8, CYP2C19, CYP2E1, or CYP3A4. Posaconazole (NOXAFIL®), fluconazole (DIFLUCAN®) and voriconazole (VFEND®), which, like ketoconazole, are known to inhibit CYP3A, did not inhibit metabolism of CMX001, indicating their CYP inhibitory activity does not extend to CYP4F2.

In conclusion, these data indicate that CYP-mediated metabolism of CMX001 is primarily by CYP4F2, and that other CYP enzymes previously identified as having a potential role in CMX001 metabolism, namely 3A4, 2C8, 2C19 and 2E1, likely do not contribute significantly to the metabolism of CMX001. The results from this study confirm the dual inhibition of CYP3A and CYP4F2 by ketoconazole, and extend the list of compounds known to inhibit CYP4F2 to include quercetin, tranylcypromine, and disulfiram. By contrast, posaconazole, fluconazole and voriconazole were not observed to inhibit CYP4F2 at concentrations below or up to 2x above the reported peak human plasma concentration for each drug; therefore, no clinically relevant interaction is expected between CMX001 and these azoles.



Objective

- To identify the CYP enzyme(s) responsible for metabolizing CMX001

Materials & Methods

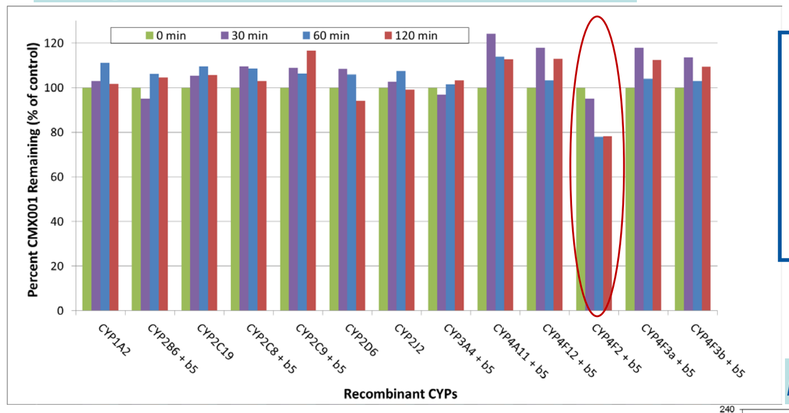
CMX001 (1 µM) was incubated with pooled human liver microsomes (HLM) and recombinant human CYP enzymes (rCYP), in the presence and absence of CYP inhibitors (XenoTech, LLC, Lenexa, KS). Concentrations of CMX001 were measured using liquid chromatography-tandem mass spectroscopy (LC-MS/MS) analysis (Tandem Labs-RTP, Durham, NC).

Background

- Previous in vitro studies identified CYP3A4, 2C8, 2C19 and 2E1 as the CYP enzymes responsible for CMX001 metabolism, based on the observed inhibition of CMX001 microsomal metabolism by direct-acting inhibitors historically recognized as specific inhibitors of CYP2C8 (quercetin), CYP2C19 (tranylcypromine), CYP2E1 (disulfiram) and CYP3A4 (ketoconazole) (Ogilvie 2008).
- A key experimental difference employed in this study was the expansion of CYP enzymes studied to include those known to metabolize medium- to long-chain fatty acids which are structurally analogous to CMX001 (including CYP2J2, 4A11, 4F2, 4F3a, 4F3b and 4F12).

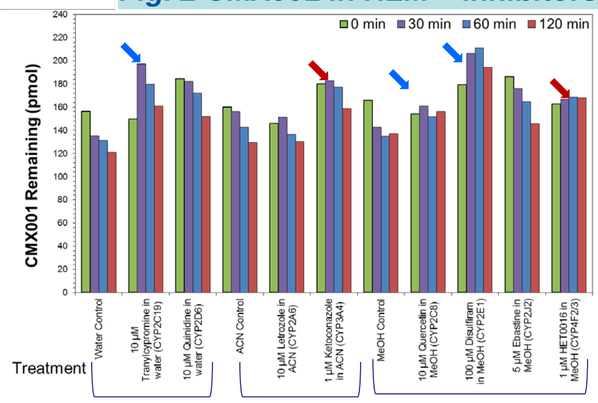
Results

Fig. 1 CMX001 in Recombinant CYPs



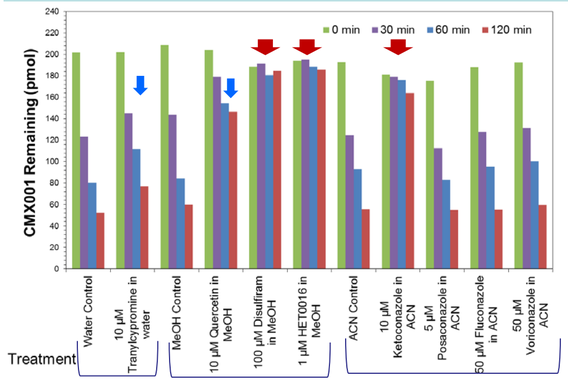
- *Incubations in this experiment with recombinant human CYP enzymes suggested that only CYP4F2 metabolizes CMX001 (red circle).*
- *No appreciable (≤ 10%) metabolism was observed in the presence of the other CYP enzymes evaluated.*

Fig. 2 CMX001 in HLM + Inhibitors



- *Inhibition of CMX001 metabolism in HLM by HET0016 (a CYP4F2/3 inhibitor) and ketoconazole (a CYP3A and CYP4F2 inhibitor) (Wang 2006) supports the rCYP results, suggesting that CYP4F2 is the primary CYP involved in the metabolism of CMX001 (red arrows).*
- *Near complete inhibition of CMX001 metabolism by quercetin, tranylcypromine and disulfiram in human liver microsomes was also observed (blue arrows).*

Fig. 3 CMX001 in Recombinant CYP4F2 + Inhibitors



- *Near complete inhibition (greater than 90%) of rCYP4F2-mediated CMX001 metabolism was observed in the presence of disulfiram, ketoconazole and HET0016 (broad red arrows).*
- *Partial inhibition (greater than 25%) of CMX001 metabolism was observed with quercetin and tranylcypromine (narrow blue arrows).*
- *Posaconazole, fluconazole and voriconazole, did not inhibit CYP4F2-mediated metabolism of CMX001 at any concentration tested up to 2x above the reported peak human plasma concentration (NOXAFIL®, DIFLUCAN®, and VFEND® product labels).*

Conclusions

- Collectively, incubations with human liver microsomes and recombinant human CYP enzymes indicate that CYP-mediated metabolism of CMX001 is primarily by CYP4F2.
- The results from this study (Fig. 2 and Fig. 3) confirm the dual inhibition of CYP3A and CYP4F2 by ketoconazole (Wang 2006), and extend the list of compounds observed to inhibit CYP4F2 to include quercetin, tranylcypromine, and disulfiram.
- CYP enzymes previously identified as having a potential role in CMX001 metabolism, namely 3A4, 2C8, 2C19 and 2E1, likely do not contribute significantly to the metabolism of CMX001.
- Accordingly, CMX001 has no risk for DDI with drugs that modulate the activity of CYP3A, 2C8, 2C19 and 2E1, nor any of the other major drug metabolizing enzymes, including CYP1A2, 2B6, 2C9, and 2D6.
- In contrast to ketoconazole, other azoles (namely, voriconazole, fluconazole and posaconazole) were not observed to inhibit CYP4F2 at concentrations below or up to 2x above the reported peak human plasma concentration for each drug; therefore, no clinically relevant interaction is expected between CMX001 and these azoles. These findings are particularly relevant to anticipated clinical use of CMX001 since these azoles are common concomitant medications in the CMX001 patient population.

References

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