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Role of Epoxide Hydrolases and Cytochrome P450s on Metabolism of KZR-616, a First-in-Class Selective Inhibitor of the Immunoproteasome

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PURPOSE

KZR-616 is a tripeptide ketoepoxide-based selective inhibitor of the human immunoproteasome. It is an analog of carfilzomib (KYPROLIS™), which inhibits all forms of the proteasome and is FDA/EMA approved for the treatment of multiple myeloma. Inhibition of the immunoproteasome blocks cytokine production in multiple immune cell types, reduces the activity of inflammatory T-helper cell subsets, increases the number of regulatory T-cells, and blocks plasma cell numbers and autoantibody production. Based on promising therapeutic activity in animal models of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), KZR-616 is being developed for potential treatment of multiple autoimmune and inflammatory diseases. KZR-616 currently is being evaluated in a Phase1b/2 clinical trial in patients with SLE and lupus nephritis. The aim of this study was to identify the major enzymes involved in the metabolism and elimination of KZR-616 in humans. The information gained will help to understand its pharmacokinetic (PK) variability and drug-drug interaction potential.

METHOD(S)

1) Metabolic stability of KZR-616 in liver microsomes (LMs) was assessed in the presence and absence of NADPH and intrinsic clearance (Clint) was monitored by the rate of parent compound disappearance.
2) Epoxide hydrolysis of KZR-616 by recombinant epoxide hydrolases (EH) was conducted at pH 9.0 for microsomal EH (mEH) and pH 7.5 for soluble EH (sEH). Cis-stilbene oxide (cis-SO) and trans-stilbene oxide (trans-SO) were used as probe substrates for mEH and sEH, respectively.
3) The kinetics of KZR-616 metabolism was studied in female and male HLMs, recombinant human mEH and human hepatocytes.
4) The effect of inhibition of EH and CYP activity by KZR-616 in HLM and/or human hepatocytes on KZR-616 clearance was performed using known inhibitors: 2-Nonylsulfanyl-propionamide (NSPA), 1-trifluoromethoxypheny1-3(1-propionylpiperidin-4-yl) urea (TPPU), and 1-aminobenzotriazole (1-ABT), for mEH, sEH and CYP activity, respectively.

RESULT(S)

1. To study if CYP or/and EH plays a role in metabolizing KZR-616

Table 1. *In vitro* metabolic stability of KZR-616 in LM

Species	HLMs		MLMs	
Cofactor (NADPH)	(-)	(+)	(-)	(+)
T1/2 (min)	194 ± 14	76 ± 4	97 ± 12	16 ± 0.4
Clint (ul/min/mg)	14 ± 1.0	36 ± 0.8	28.6±3.6	173 ±.4.6

As shown in above Table 1, the CLint increased by 2.6 and 6 folds for HLMs and MLMs, respectively, in the presence of NADPH comparing with that in the absence of NADPH, suggesting CYP activity may play a role in the metabolism of KZR-616, which is not consistent with what we found in the in vivo studies.

2. To confirm the role of CYP on KZR-616 metabolism using a pan CYP inhibitor (1-ABT) in human hepatocytes.

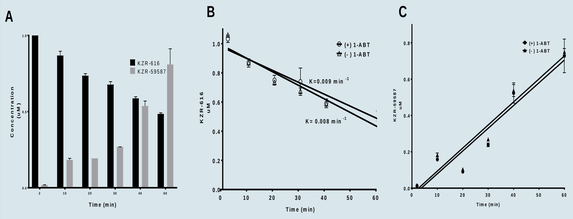
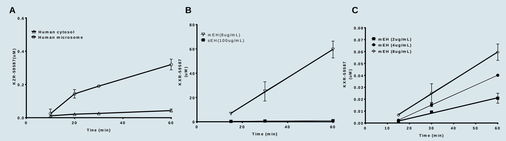


Figure 1. KZR-616 metabolism in human hepatocytes

As shown in above Figure 1:

- A pan CYP inhibitor (1-ABT) does not affect the elimination rate(k), indicating that CYPs did not play an important role in the metabolism of KZR-616 in human hepatocytes.
- KZR-59587, a diol form of KZR-616, was found to be the major metabolite in hepatocytes.

3. KZR-616 epoxide hydrolysis pathway study



The diol KZR-59587 was resulted from direct epoxide hydrolysis of KZR-616. The epoxide hydrolysis of KZR-616 was therefore investigated in human liver microsomes (mEH), hepatic cytosols (sEH) and recombinant EHs. As shown in Figure 2:

- Formation of KZR-59587 increased linearly with increasing liver microsomal proteins, suggesting it formed via mEH;
- No formation of KZR-59587 was observed when KZR-616 was incubated in human hepatocyte cytosol, indicating its formation was not from sEH.
- Using the recombinant enzyme mEH or sEH, KZR-59587 was confirmed to be formed via mEH.

4.Inhibition of KZR-616 epoxide hydrolysis in HLMs by a selective mEH inhibitor (NSPA)

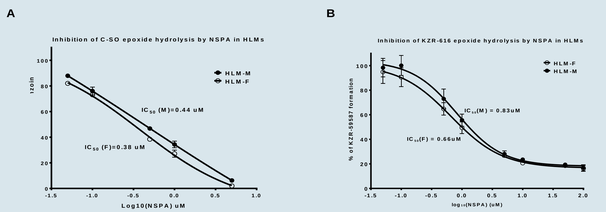


Figure 3. KZR-616 epoxide hydrolysis was inhibited by NSPA, a selective mEH inhibitor, confirming mEH plays a significant role in KZR-616 metablism.

5. Inhibition of KZR-616 epoxide hydrolysis in human hepatocytes by mEH inhibitor NSPA and sEH inhibitor TPPU

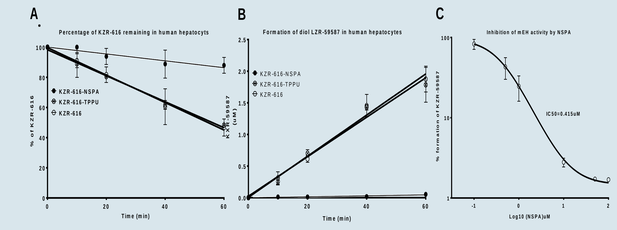


Figure 4. The formation of KZR-59587 was significantly affected by addition of mEH inhibitor NSPA with IC50 of 0.42 μM, but not affected by sEH inhibitor TPPU, confirming the predominant KZR-616 metabolic pathway is via mEH hydrolysis.

6. Investigation of KZR-616 as inhibitor on epoxide hydrolases in liver microsomes, cytosol and recombinant EHs.

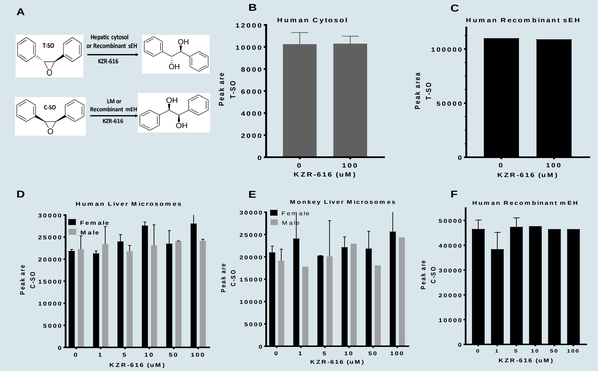


Figure 5 data shows that KZR-616 did not inhibit cis-SO and trans-SO hydrolysis in either human, monkey LM, hepatic cytosols or with recombinant EHs at concentrations up to 100μM, suggesting that KZR-616 is unlikely to cause clinically drug-drug interaction.

CONCLUSION(S)

- KZR-616 metabolism predominantly driven by mEH
- CYP450 and sEH play little/no role in metabolism
- Hepatocytes serve as a good in vitro system to assess the metabolic profiles of KZR-616
- PK of KZR-616 is unlikely to be affected by co-administration of CYP450 and sEH inhibitors/inducers
- KZR-616 is unlikely to alter epoxide hydrolysis of other mEH and sEH substrate drugs.

REFERENCE

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