Contributions of Mitochondrial Damage and Hepatocyte Toxicity to Pexidartinib-Induced Hepatotoxicity

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Abstract

Background: Pexidartinib is a recently approved small molecule kinase inhibitor (KI) to treat adults with tenosynovial giant cell tumor. Pexidartinib has a boxed warning for hepatotoxicity and, thus, is available only through the FDA Risk Evaluation and Mitigation Strategy (REMS) program. **Purpose:** Since mitochondrial liability and direct hepatocyte cytotoxicity have been associated with KI-induced hepatotoxicity, the effects of pexidartinib on mitochondrial functions and hepatocyte viability were examined. **Methodology:** Freshly isolated rat liver mitochondria, submitochondrial fractions, and cryopreserved primary human hepatocytes (PHHs) were treated with pexidartinib at clinically-relevant concentrations, and mitochondrial function and cytotoxicity were assessed. **Results:** In isolated mitochondria, the state 3 oxygen consumption rates of glutamate/malate- and succinate-driven respiration were both decreased by pexidartinib, with the former being more profoundly inhibited. In contrast, the effect on the state 4 oxygen consumption rates was negligible, suggesting pexidartinib is not an uncoupler. In liver submitochondrial fractions, the activities of respiratory chain complex (RCC) I and V were significantly inhibited by pexidartinib, with greatest potency at RCC I; whereas complexes II, III, and IV were unaffected. In PHHs, pexidartinib decreased the adenosine triphosphate (ATP) level, increased the reactive oxygen species level, and caused cell death. However, caspase activities were unaffected, suggesting pexidartinib does not affect apoptosis. Seahorse analysis with PHHs showed that mitochondrial respirations, but not the rate of glycolysis, were inhibited by pexidartinib prior to apparent cell death. All the effects noted above occurred at pexidartinib concentrations of 0.5- to 2.5-fold the human peak blood concentration (Cmax) achieved with the recommended therapeutic dose. **Conclusion:** Taken together, these data suggest that pexidartinib selectively inhibits mitochondrial respiratory chain complex I and V, which causes ATP depletion and oxidative stress and leads to hepatocyte death. Therefore, mitochondrial injury and the resulting hepatocyte cytotoxicity are implicated in the mechanism of pexidartinib-induced hepatotoxicity.

Introduction

Pexidartinib Background:

- A kinase inhibitor approved by FDA on April 2, 2019
- Targets colony stimulating factor 1 receptor (CSF1R), KIT proto-oncogene receptor tyrosine kinase (KIT), and FMS-like tyrosine kinase 3 (FLT3). (Not expressed in normal hepatocytes)
- Indicated for treating tenosynovial giant cell tumors (TGCT), a type of benign tumors
- First and only FDA-approved treatment for TGCT
- May be chronically administered (up to years)
- Strongly hepatotoxic: a boxed warning for hepatotoxicity is included in its labeling (“can cause serious and potentially fatal liver injury”)
- “Available only through a restricted program called the TURALIO Risk Evaluation and Mitigation Strategy (REMS) Program”

Published data show that kinase inhibitors with boxed warnings for hepatotoxicity are either mito-toxic (4 out of 6; 67%), cytotoxic to hepatocytes (6 out of 6; 100%), or both.

Hypothesis: Pexidartinib is mito-toxic and cytotoxic to hepatocytes

Materials and Methods

- Isolated hepatic mitochondria and commercial human primary hepatocytes (PHHs) were treated with pexidartinib at concentrations normalized to blood levels at a therapeutic dose (Cmax = 20.6 μM; the highest among 66 FDA approved kinase inhibitors)
- Toxicity endpoints: mitochondrial oxygen consumption rate, respiratory chain complex (RCC) activities, cellular ATP levels, levels of reactive oxygen species (ROS), mode of cell death: necrosis (CCK-8 assay) vs. apoptosis (caspase 3/7 assay)

Results

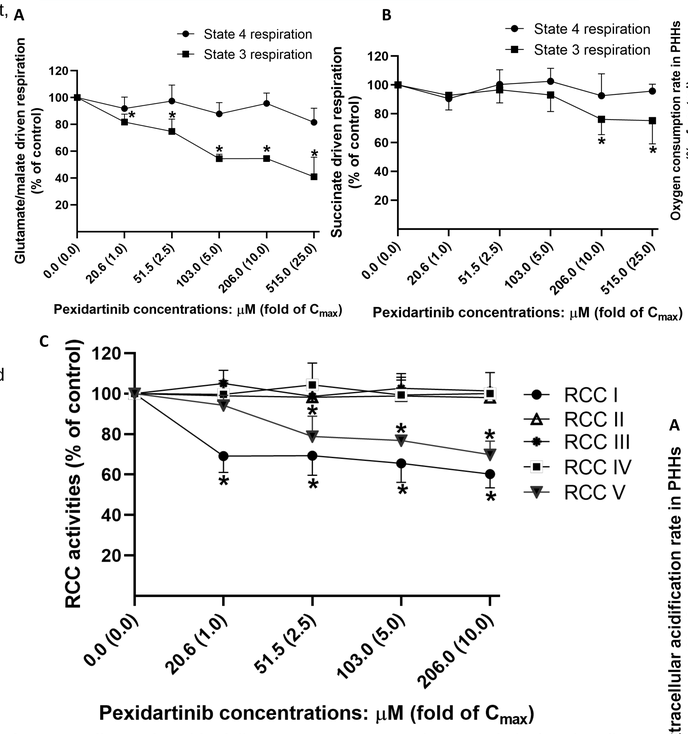

Figure 1. Effects of pexidartinib on oxygen consumption rates in isolated rat liver mitochondria (A and B) or RCC I-V activities (C) in submitochondrial fractions. Freshly isolated rat liver mitochondria or submitochondrial fractions were adjusted to 1 mg/ml protein, treated with pexidartinib or vehicle control (0.1% DMSO) for 5 min, and mitochondrial oxygen consumption or RCC I-V activities were measured. The signal in pexidartinib-treated groups were normalized to DMSO-treated vehicle control groups set to 100% activity. The glutamate/malate and succinate-driven respiration rates are shown in panels A and B, respectively. * $p < 0.05$ compared to DMSO-treated samples.

Results

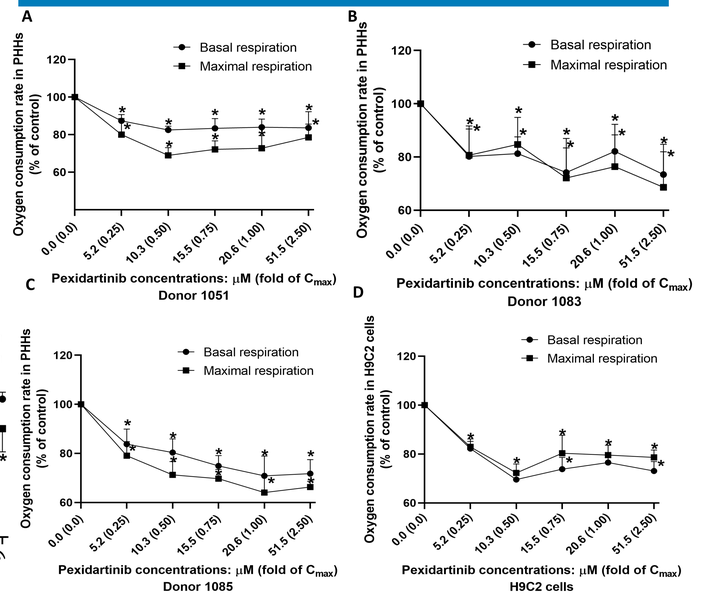

Figure 2. Effects of pexidartinib on oxygen consumption rates in PHHs (A to C) and H9C2 cells (D). Cells were treated with pexidartinib for 1 h and then oxygen consumption rates were measured. The signal in pexidartinib-treated groups were normalized to DMSO-treated vehicle control groups set to 100%. * $p < 0.05$ compared to DMSO-treated samples.

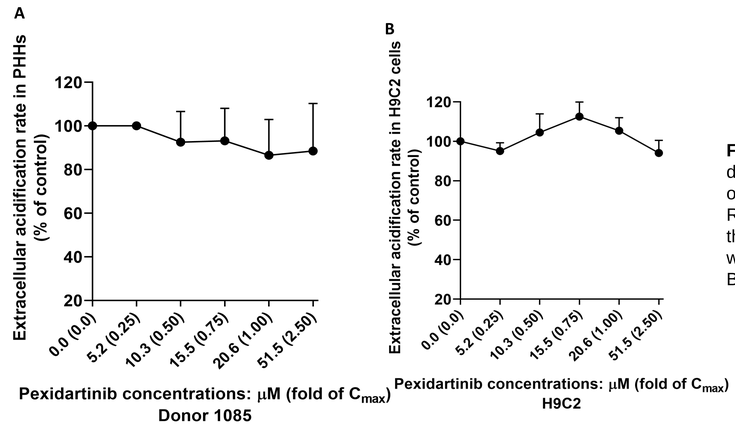

Figure 3. Effects of pexidartinib on extracellular acidification rate in PHHs (A) and H9C2 cells (B). Cells were treated with pexidartinib for 1 h and then extracellular acidification rate were measured. The signal in pexidartinib-treated groups were normalized to DMSO-treated vehicle control groups set to 100%.

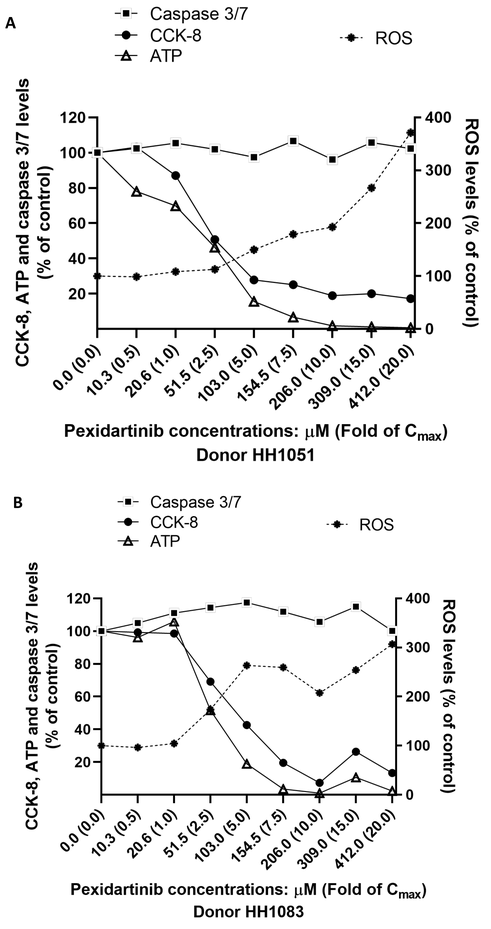

Figure 4. Cytotoxicity of pexidartinib in PHHs. PHHs from two different donors, HH1051 and HH1083, were treated with pexidartinib or vehicle control (0.1 % DMSO) for 24 h, and the cellular ATP and ROS levels and caspase 3/7 activities were determined. Additionally, the numbers of live cells were measured using CCK-8 assay. Data were normalized to vehicle control groups set to 100%. Panels A and B represent data from each donor.

Conclusion

Pexidartinib directly inhibits RCC I and V in submitochondrial fractions isolated from rat liver, and causes ATP shortage, oxidative stress and subsequent cell death in PHHs. These detrimental effects are likely to contribute to the pathogenesis of pexidartinib-induced hepatotoxicity.