Investigation of isoniazid DILI mechanisms in human induced pluripotent stem cell derived hepatocytes

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Abstract

Isoniazid (INH), the preferred medication for prevention and treatment of tuberculosis, has been associated with idiosyncratic drug induced liver injury (IDILI). INH causes benign ALT elevations (> 3 X ULN) in about 10% patients, and causes clinically important DILI in about 0.1% patients. Formation of INH-protein adducts in human liver microsomes, detection of antibodies targeting INH and INH-adduted proteins in IDILI patients serum, and positive lymphocyte transformation test (LTT) in IDILI patients all supported the hypothesis that INH-induced IDILI is adaptive immune mediated. But the mechanism for these rare immune reactions remain unclear. It is widely speculated the genetic factors contribute to individuals' susceptibility towards INH hepatotoxicity. In the current study, hepatocytes derived from induced pluripotent stem cells (iPSC), which carry the donor's genetic information, were characterized for studying INH hepatotoxicity. The Vmax of CYP2E1 in iPSC-derived hepatocytes were 16.0 ± 3.0 or 10.8 ± 2.2 pmol/min/10^6 cells using 4-Nitrophenol or chlozoxazone as substrate, respectively. Parallel experiments in primary human hepatocytes (pHH) were performed with 4-Nitrophenol and activity was calculated as 21.2 ± 0.5 pmol/min/10^6 cells. INH-adducted proteins were assessed by western blot using rabbit anti INH antiserum. Both iPSC-derived hepatocytes and pHH had dose-dependent INH-protein adducts formation, in which iPSC-derived hepatocytes had a higher amount. Major bands of INH-adducted proteins in both cell types were qualitatively similar, but there were quantitative differences. Viability of iPSC-derived hepatocytes after various concentrations of INH treatment were assessed by measuring cellular ATP content or Lactate dehydrogenase (LDH) released in to the culture medium. INH, up to 10 mM, which is 100 fold higher than the C_{max} in patients serum, do not cause noticeable ATP loss, LDH release or morphological changes in iPSC-derived hepatocytes. However, an increase in the release of microparticles into the medium was observed in iPSC-derived hepatocytes with 5 mM INH treatment. The majority of the particles sized around 0.1 μm with minimum side scatter readings. Untreated control cells have much fewer particles released. At non-cytotoxic concentrations, INH also induced concentration-dependent decrease of oxygen consumption rate as well as mitochondrial membrane potential as measured by Seahorse and high content imaging, respectively. In summary, the iPSC-derived hepatocytes exhibited CYP2E1 activity comparable to primary hepatocytes. At non-cytotoxic INH concentrations, iPSC-derived hepatocytes formed INH-adducted proteins and released microparticles, which may serve as a mechanism of neoantigen presentation as well as release of "danger signals" required for lymphocyte activation.

Materials and Methods

iPSC-derived hepatocytes (iCell® Hepatocytes) were obtained from Cellular Dynamics International (CDI). Primary human hepatocytes were obtained from Triangle Research Labs (TRL). iPSC-derived hepatocytes and primary human hepatocytes were plated according to their recommended protocols. All cells were plated at a density of 60K/well (96-well plate), 400K/well (24-well plate) or 2M/well (6-well plate) on collagen coated plates and incubated for 24h. Then all experiments were performed in HMM medium supplemented with penicillin, ITS+ and 0.1uM dexamethasone. Concentrations of CYP2E1 metabolites, 4-nitrocatechol and 6-hydroxychlorzoxazone were determined by SpectraMax microplate reader and LC/MS/MS (QPS), respectively. INH addicted proteins were blotted with anti-INH antibody (provided by Dr. Uetrecht, University of Toronto), and identified by LC/MS/MS proteomic analysis. ATP and LDH levels were measured using Cell-Titer Glo™ and CytoTox-One™ assays (Promega). Release of microparticles were measured in BD FACSCanto™ II Flow Cytometer.

Results

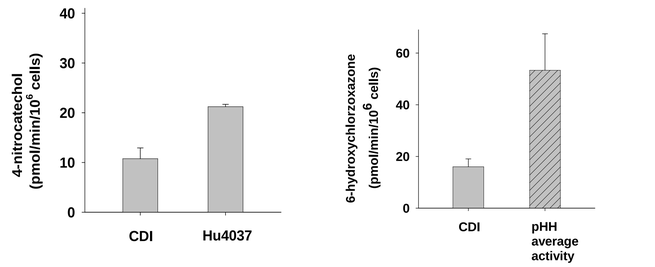

Figure 1. iPSC-derived hepatocytes exhibit CYP2E1 activity. Vmax of CYP2E1 catalyzed O-hydroxylation of *p*-nitrophenol (left) and 6-hydroxylation of chlorzoxazone (right) were measured in iHC and primary human hepatocytes. For 4-nitrocatechol experiment, Hu4037 batch of primary human hepatocytes were measured in parallel with iPSC-derived hepatocytes. For 6-hydroxyl chlorzoxazone, average activity of primary human hepatocytes were obtained from the literature.

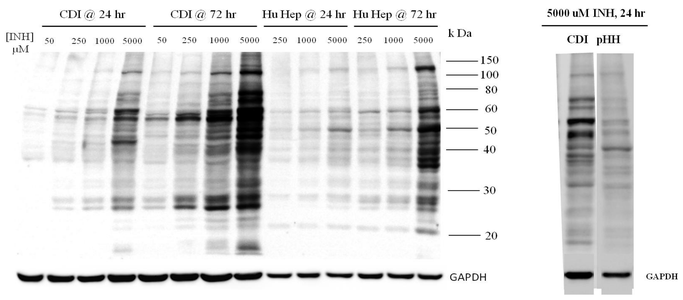

Figure 2. INH-protein adducts were formed similarly in iPSC-derived hepatocytes (CDI) and primary human hepatocytes. iHC and primary human hepatocytes were incubated with increasing doses of INH for 24h and 72h. INH protein adducts in whole cell lysates were blotted with an anti-INH antibody.

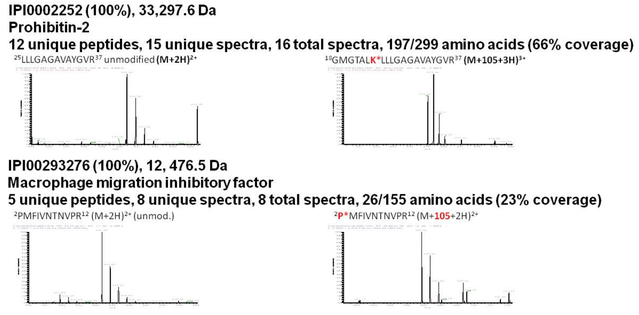

Figure 3. Proteomic identification of INH-adducted proteins in iPSC-derived hepatocytes. iPSC-derived hepatocytes were incubated with 5mM INH for 72h. Total cell lysate was separated by SDS-PAGE, digested with trypsin and analyzed by LC-MSMS. The results were searched against the IPI (human) database for protein identities and the presence of INH-adducts.

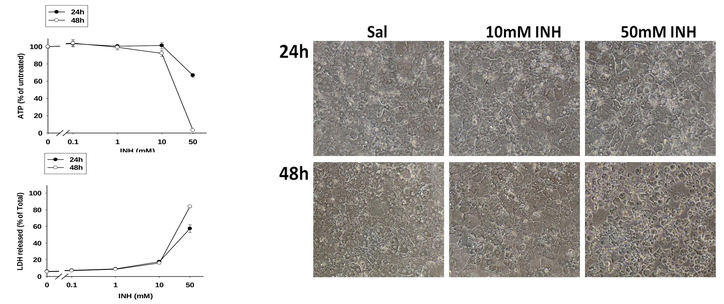

Figure 4. Concentrations of INH exceeding 2 orders of magnitude the patient Cmax are not overtly toxic. iPSC-derived hepatocytes were treated with increasing concentrations of INH for 24h or 48h. Left, INH-induced ATP drop and LDH release after 24h (black circles) or 48h (white circles) incubation. Right, representative graphs of iPSC-derived hepatocytes after INH incubation.

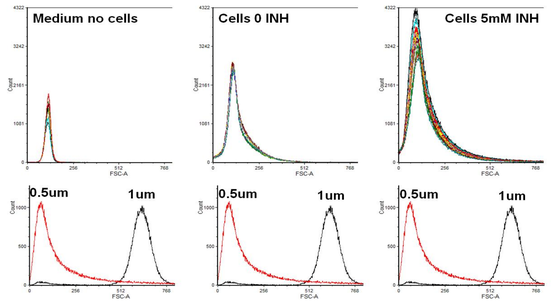

Figure 5. Nontoxic doses of INH caused CDI cells to release microvesicles. iPSC-derived hepatocytes were treated with 0 or 5mM INH for 72h. Top, forward scatter histogram for microparticles in medium from: No cells (left), untreated cells (middle) and 5mM INH-treated cell (right). Bottom, latex marker beads were used as size standards (0.5μm, red line; 1μm, black line).

Conclusion

Drug → 1). Reactive metabolites → 2). neoantigens, 3). "danger signal" → 4). adaptive immune attack

1. iPSC-derived hepatocytes have CYP2E1 activity.
2. iPSC-derived hepatocytes generate INH-protein adducts that are qualitatively similar to those produced by primary human hepatocytes . LC/MS/MS proteomic analysis identified prohibitin-2 and macrophage migration inhibitor factor as INH-adducted proteins in iPSC-derived hepatocytes.
3. INH exceeding 2 orders of magnitude patient Cmax did not cause overt cytotoxicity in iPSC-derived or primary human hepatocytes
4. Nontoxic doses of INH caused release of ~0.5μm microparticles in iPSC-derived hepatocytes
5. iPSC-derived hepatocytes prepared from patients who have experienced INH DILI are promising models for additional mechanistic studies.