A fluorescent agent cocktail for detecting both cholestasis and hepatocellular forms of acute drug-induced liver injury

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Abstract

Drug induced liver injury (DILI) is a major reason for late stage termination of drug discovery research projects, so assessment is being integrated earlier in the drug development process. Some chemicals can produce different forms of hepatic injury in mice, including the two most common forms, cholestasis and hepatocellular injury. Biochemical serum markers of liver damage, like alanine transaminase (ALT) and alkaline phosphatase (ALP) are limited in their ability to detect both of these common forms of liver injury and focus on plasma as an indirect measure of what is occurring in the liver.

In contrast, in vivo fluorescent imaging offers non-invasive detection of a variety of biological changes detected directly in liver tissue, and high throughput epifluorescence (3-5 mice at a time) compared favorably to the more accurate acquisition of individual animal morphologic datasets. We assessed three different near infrared fluorescent imaging agents, specific for cell death (Annexin-Vivo 750 (AV750), matrix metalloproteases (MMPs)65 FAST), and transferrin receptor (Transferrin-Vivo 750) and discovered different patterns of liver responses among these different agents after dosing with L-Sorbinol. No combination of agents was selected that did not induce hemorrhage within the liver, as assessed by vascular agent leakage, except the persistence of passive agent accumulation within the liver. In addition, agents were injected at different timepoints post-drug to account for potential differences in the kinetics of the liver injury induction. A single dose of hepatocellular injury-inducing agents, thiacetamide (TAA, 300 mg/kg) and acetylamidophenol (AAP, 300 mg/kg), showed late induction of acute injury (18-24h), the liver cholestasis-inducing drug rifampicin (RMP, 350 mg/kg) showed a very early liver response, and chlorpromazine (CPZ, 100 mg/kg), which induces mixed hepatocellular/cholestasis injury, showed both early and late signal.

By using a cocktail of these three near infrared fluorescent imaging agents, all labeled with 750 nm fluorophores, each of the four different drugs showed comparable total fluorescent signal within the liver region by epifluorescence imaging. A strategy of agent cocktail injection in separate cohorts at 2h and at 2h allowed the effective detection of drugs with either early- or late-onset injury. Compared to conventional plasma/serum assays, in vivo imaging can offer a variety of advantages for drug discovery, such as early detection of drug effects and more accurate assessment of the tissue of interest. Our results to date demonstrate the potential of optical imaging for assessing the potential for compound liver toxicity in early drug discovery programs.

1 Liver Injury Imaging Protocol

DILI Model Experimental Description: Mouse Acute Liver Injury Screening Model

Revised BALb/c or C57BL6 mice (Charles River Laboratories) were injected IP with DILI-inducing drugs. At 2 or 18h, mice were injected with imaging agents to detect biological changes within damaged liver tissue.

2 Liver Imaging Approaches

Optical Imaging Modes:

- Transillumination (3D)
- Tomography (2D)
- Fluorescence
- Epifluorescence

3 Imaging Agent Profiling of DILI

A. Hepatocellular Liver Injury Agents: IVIS Spectrum CT Images

Control vs. drug administered mice at 2, 24h post-drug with AV750, MMPI45, and TAA/750 in the liver: different colored liver regions of mice at different times post-treatment. B. Cholestasis and Mixed Liver Injury Agents: IVIS Spectrum CT Images

Control vs. drug administered mice at 2, 24h post-drug with AV750, MMPI45, and TAA/750 in the liver: different colored liver regions of mice at different times post-treatment. C. Epifluorescence Analysis

Fluorescence images of mice receiving optimal dosages of control, RMP, CPZ, and TAA show differences in liver injury, compared with control mice. D. Imaging of 3 agents for DILI screening in vivo

Whole mouse epifluorescence imaging was used to detect accumulation of AV750, MMPI45, and TNC750 in the liver of different cohorts of mice at different times post-treatment. A. Early and Late Liver Injury Imaging with Compensation for Vascular Leak

A. IVIS Spectrum CT Images

Control vs. drug administered mice at 2, 24h post-drug with AV750, MMPI45, and TAA/750 in the liver: different colored liver regions of mice at different times post-treatment. B. Cholestasis and Mixed Liver Injury Agents: IVIS Spectrum CT Images

Control vs. drug administered mice at 2, 24h post-drug with AV750, MMPI45, and TAA/750 in the liver: different colored liver regions of mice at different times post-treatment. C. Epifluorescence Analysis

Fluorescence images of mice receiving optimal dosages of Angiostein 680 (upper panel), 750 nm cocktail (middle panel) in controls or at best times post-treatment. The upper Panel shows normalized images generated on the IVIS Spectrum CT Living Image 4.5 software. Quantification of liver signal from non-invasive imaging was used to detect accumulation of the 680 nm and 750 nm agents in the liver regions of different cohorts of mice post-treatment. Different agents show the benefit of incorporating both 2h and 24th timepoint imaging with RMP showing earlier liver injury and TAA showing later onset of injury.

Summary

The present study provides evidence for the utility of a cocktail of imaging agents, detecting cell death, MMP activity, and transferrin receptor upregulation, in the detection of acute drug-induced liver injury. Whereas, the four unique drugs tested showed different patterns of these biological changes, the imaging agent cocktail (all agents at 750 nm) was designed to detect all three biological changes simultaneously, as a universal imaging agent for detecting liver injury in the absence of any knowledge of a specific drug’s mechanism of liver injury. A strategy of looking at both an early timepoint (2h) and a later timepoint (24h) helped to minimize the chance of false negative results. Further incorporation of a vascular agent (Angiostein 680) on another imaging channel allowed both the collection of vascular leak information and the ability to compensate for non-specific liver accumulation of the 750 nm cocktail.