Closing the gap on liver toxicity

By Tim Fulmer, Senior Writer

U.S. researchers have identified a small molecule gap junction inhibitor that protects mice from drug-induced liver toxicity.[1] The team has founded **Heprotech Inc.** to further characterize and optimize the inhibitor.

Gap junctions are multiprotein channel structures that directly connect the cytoplasm of two adjacent cells, allowing for electrical and chemical communication.

In previous cell culture studies, Martin Yarmush and colleagues showed that gap junctions play a role in the propagation of inflammatory and antiviral signals between several cell types including hepatocytes.[2] Yarmush is professor of surgery at **Harvard Medical School** and **Massachusetts General Hospital**.

The pathogenesis of drug-induced liver injury (DILI) involves proinflammatory cellular damage propagating from an initial injury site to an increasingly larger area of the liver.[3] DILI is most commonly caused by an overdose of the analgesic acetaminophen, and the only drug approved to counteract that process is N-acetylcysteine (NAC).

NAC is most effective when given within eight hours of an acetaminophen overdose. Standard treatment durations are 72 and 48 hours for the oral and i.v. formulations, respectively.[4]

Yarmush and colleagues hypothesized that selectively blocking gap junctions in the liver might help limit DILI or at least slow its progression.

To test that hypothesis, the researchers generated mice deficient in gap junction protein β1, 32 kDa (Gjb1; Cx32; connexin-32), the main gap junction protein in the liver, and treated them with a hepatotoxin called thioacetamide that is known to cause liver failure.

The Cx32 knockout mice receiving thioacetamide had less liver damage and a lower hepatic inflammatory response than wild-type mice given the same toxin. In the knockout mice, liver tissue showed less recruitment of neutrophils and lower levels of multiple proinflammatory cytokines including tumor necrosis factor-α (Tnf-α), Il-6 and chemokine CC motif ligand 5 (Rantes; Ccl5).

Moreover, all knockout mice survived a lethal dose of the toxin, whereas all wild-type mice died.

Next, the researchers used cell-based assays to screen libraries for small molecule inhibitors of CX32. The top hit was the small molecule 2-aminoethoxydipenyl borate (2APB), a research tool previously shown to transiently bind and inhibit CX32 gap junctions *in vitro*.[5]

To test whether 2APB also decreases DILI *in vivo*, the team turned to mouse models of thioacetamide- and acetaminophen-induced liver injury. Indeed, 2APB given 1.5 hours after chemical challenge reduced hepatotoxicity compared with vehicle control, as measured by serum liver enzymes and liver histology.

2APB also lowered serum liver enzyme levels and limited further hepatocellular damage and necrosis compared with vehicle when given six hours after chemical challenge, which is after the onset of hepatic necrosis.

Data were published in *Nature Biotechnology*.

The authors wrote that 2APB and similarly acting compounds "may provide a clinically useful means to treat liver injury associated with dose-dependent hepatotoxic drugs such as acetaminophen."

"The strengths of this study are that it uses two models of DILI—thioacetamide and acetaminophen—thus supporting the idea that a number of investigators have been building upon that innate immune activation is a feature common to many forms of hepatocyte death and thus common to many agents that cause DILI," said Wajahat Mehal, associate professor of medicine at **Yale University**.

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> ***—Anthony Phillips, CoDa Therapeutics Inc.***

In 2009, Mehal and colleagues published data on mouse models of DILI showing that DNA released from dying hepatocytes activates the innate immune response via toll-like receptor 9 (Tlr9) and the NLR family pyrin domain containing 3 (Nlrp3; Nalp3) inflammasome.[6]

"The advantage of a CX32 inhibitor is that it acts at a later stage in the acetaminophen hepatotoxicity process than N-acetylcysteine" and thus could have a wider therapeutic window, said Mehal.

Minding the gap

Going forward, Mehal wanted to see the gap junction inhibitor tested in additional animal models of acetaminophen-induced liver toxicity.

Andrew Harris, professor of pharmacology and physiology at the **UMDNJ New Jersey Medical School**, said it will be important to see whether 2APB has any effects in tissues outside the liver, because gap junction proteins are expressed throughout the body. For example, the proteins are essential for proper cardiac function.

In 2007, Harris published research showing that 2APB inhibited CX32 and gap junction protein β2, 26 kDa (GJB2; connexin-26; CX26), a gap junction protein expressed in the liver and other tissues.[4]

Those data "raise some questions about the specificity of 2APB," said Anthony Phillips, medical director of **CoDa Therapeutics Inc.** "It will be important to look at the potential effects of 2APB on other connexin proteins as well as consider the proper length of treatment to achieve the optimal therapeutic effect."

CoDa has two compounds in development that target gap junction proteins. Lead molecule Nexagon is a topically delivered

oligonucleotide that targets gap junction protein α1, 43 kDa (GJA1; CX43; connexin-43). The compound is in Phase II testing to treat chronic venous leg ulcers. The company also has a peptide mimetic that targets gap junction proteins in preclinical development for undisclosed indications.

CoDa cofounder David Becker told *SciBX* that "to the best of our knowledge, there are no small molecules that are totally specific for a particular gap junction protein. That's a key reason why CoDa went the antisense route to downregulate the expression of a specific connexin without any off-target effects."

Becker is a professor of cellular imaging at the **University College London**.

The findings in the paper are covered by patents licensed to Heprotech, a company founded by corresponding author Yarmush and lead author Suraj Patel, who is a research fellow in the Massachusetts General Hospital Department of Surgery.

Next steps for the company include studying any off-target effects of gap junction inhibitors and understanding the long-term effects of blocking liver-specific gap junction channels, said Yarmush. He declined to provide additional details.

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COMPANIES AND INSTITUTIONS MENTIONED

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