Plant toxin causes biliary atresia in animal model

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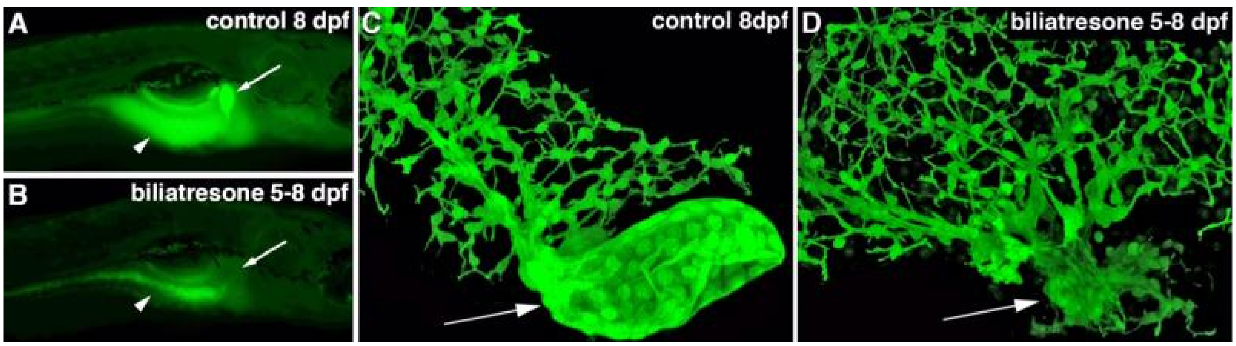


A composite showing reduced bile flow (A, B) and severe damage to the gallbladder (C, D) in an eight-day-old zebrafish larva that received a low dose of the isolated plant toxin biliatresone for three days and the normal gallbladder of a control zebrafish larva that did not receive the toxin. Arrows point to the damaged gall bladder. The other green structures are bile ducts in the liver. The ducts connecting the liver to the gallbladder are also affected by the toxin. Credit: Michael Pack, M.D., Perelman School of Medicine, University of Pennsylvania.

A study in this week's *Science Translational Medicine* is a classic example of how seemingly unlikely collaborators can come together to make surprising discoveries. An international team of gastroenterologists, pediatricians, natural products chemists, and veterinarians, working with zebrafish models and mouse cell cultures have discovered that a chemical found in Australian plants provides insights into the cause of a rare and debilitating disorder affecting newborns. This ailment, called biliary atresia (BA), is the most common indication for a liver transplant in children.

The team isolated a plant toxin with a previously uncharacterized chemical structure that causes biliary atresia in zebrafish and mammals, noted collaborators Michael Pack, MD, a professor of Medicine in the Gastroenterology Division and the department of Cell and Developmental Biology, and Rebecca Wells, MD, an associate professor of Medicine in the Gastroenterology Division and the department of Pathology and Laboratory Medicine, both at the Perelman School of Medicine, University of Pennsylvania.

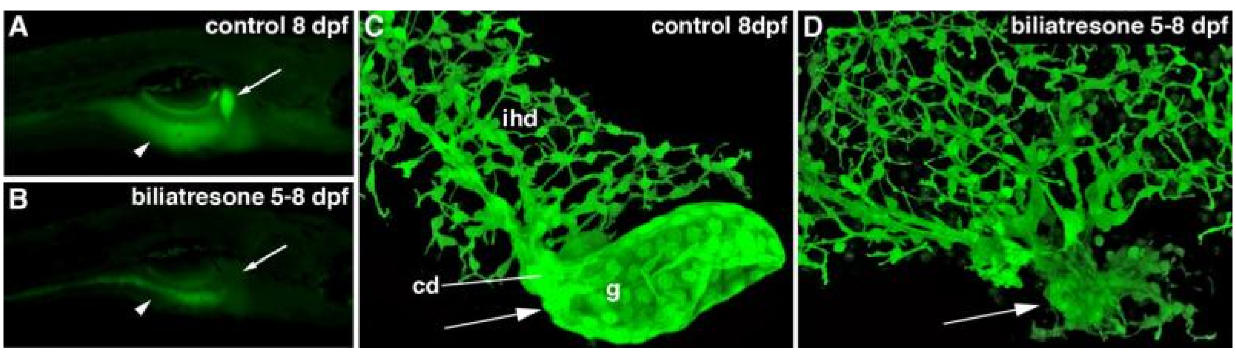
BA is a rapidly progressive and destructive disorder that affects the cells lining the extra-hepatic bile duct. The cells within this large duct, which carries bile from the liver to the small intestine, are damaged as the result of an as-yet-unidentified environmental insult, a toxin or infection, resulting in scarring (fibrosis) that obliterates the duct, thus preventing bile flow.

The incidence of BA is 1/10,000 to 15,000 live births. It occurs worldwide and is one of the most rapidly progressive forms of liver cirrhosis and liver failure. Fortunately, a life-saving treatment for BA is available for babies, a surgical procedure called a Kasai portoenterostomy, in which a small segment of intestine is connected directly to the liver, to restore bile flow. Most babies, though, eventually develop cirrhosis of the liver and ultimately liver failure, leading to the need for a transplant either in infancy, childhood or adolescence.

A healthy lamb (on left) compared to a lamb afflicted with biliary atresia (on right). The lamb afflicted with biliary atresia is smaller than its healthy counterpart and reflects a modified posture and atrophied musculature. Credit: Steve Whittaker, Hume Livestock and Pest Authority

Working with pediatric gastroenterologists Elizabeth Rand, MD and David Piccoli, MD and other colleagues at the Fred and Suzanne Biesecker Pediatric Liver Center at The Children's Hospital of Philadelphia, Pack and Wells became interested in a naturally occurring BA model reported by veterinarians in Australia. During years of extreme drought over the last four decades, sheep and cows grazed in unusual pastures had given birth to offspring with a BA-like syndrome that was essentially identical to human BA. Field veterinarian Steve Whittaker, BVSc, and veterinary scientist Peter Windsor, DVSc, PhD, who diagnosed BA in lambs during a drought in 2007, correlated the outbreaks with ingestion of plants in the genus Dysphania, including a plant called pigweed, that grew on lands normally under water, suggesting a toxic cause of the animal BA

"The 2007 drought persisted through 2008, enabling us to harvest Dysphania species plants from a pasture implicated in the 2007 episode," recalls Wells, who initiated the collaboration by contacting Whittaker soon after the 2007 outbreak and arranged to have the plant harvested and imported into the U.S. "With the plant in hand, we knew we had a chance to identify the responsible biliary toxins, guided by a zebrafish bioassay devised in my laboratory," adds Pack.



Fluorescent images of a live, normal zebrafish larva (A) and a larva treated with the toxin biliatresone (B). Gallbladder fluorescence (arrow) is absent and intestinal fluorescence (arrowheads) is markedly reduced in the toxin-treated larva. Confocal microscopy projections of the gallbladder and extrahepatic bile ducts of a control larva (C) and a larva treated with biliatresone (D) show morphological defects produced by the toxin. Credit: Kristin Lorent

Collaborator and co-author John R. Porter, PhD, a natural products chemist from the University of the Sciences in Philadelphia, made crude extracts from the plants and in an iterative process, Pack used the zebrafish bioassay to winnow this mixture containing thousands of compounds down to a mixture of four compounds.

From this mixture, the team finally isolated a previously undescribed isoflavone they called biliatresone. This toxin causes BA in five-day old larval zebrafish, selectively destroying the bile ducts outside the liver, but not inside the liver. The toxin also had no obvious effect on any other of the fish tissues - similar to the findings in human BA. Wells' group showed that the plant toxin also has significant effects on mammalian cells, causing changes in biliary cell structure and organization that mimic changes in the afflicted human bile duct.

One of the perplexing qualities of the toxin is its specificity for the large, extra-hepatic bile ducts. To better understand why other liver cell types and smaller bile ducts were not affected, Pack examined various zebrafish mutants, with the hope of finding one that might be either more sensitive or resistant to bile duct injury. Remarkably, his group was able to identify a mutant that was sensitized to the toxin, and this mutation mapped to a region in the zebrafish genome that is similar to an established human BA susceptibility region found in a previous genome-wide study. This provided further evidence that the Dysphania BA syndrome will be important for understanding human BA.

"Taken together, these findings provide direct evidence that BA could be initiated by prenatal exposure to an environmental toxin," say Pack. And while it is clear that humans do not consume the pigweed or related plants implicated in the sheep BA outbreaks, a non-toxic, structurally related compound that co-purified with biliatresone is found in beets, chard, and other consumable plants. The team is currently studying whether gut bacteria can convert this inactive compound into the active toxin.

Going forward, the researchers are attempting to synthesize a large enough quantity of the toxin to study its effects in mice, which have a liver and biliary system more closely related to humans than fish. In addition, Pack and Wells will work with the models that they have already established in their respective laboratories to determine the toxin's mechanism of action and understand how this can be used to prevent and treat BA.

More information: Identification of a plant isoflavonoid that causes biliary atresia, stm.sciencemag.org/lookup/doi/ … scitranslmed.aaa1652

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