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BILIARY TRACT

Newly identified biliatresone causes biliary atresia

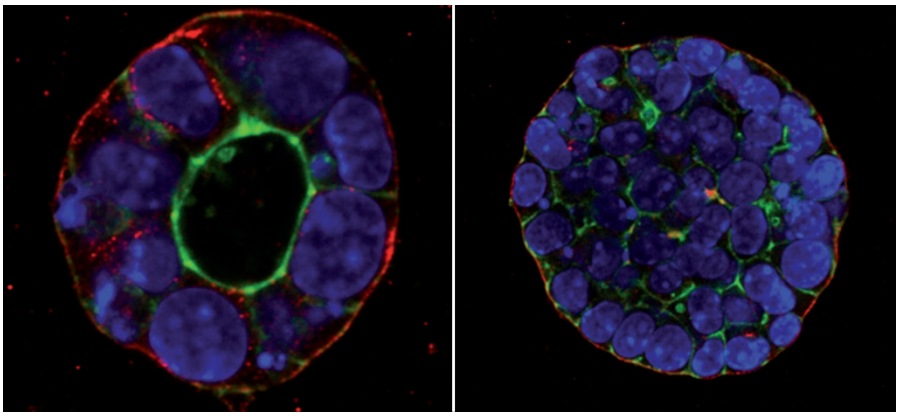
Research from Michael Pack and Rebecca Wells' groups published in *Science Translational Medicine,* has shed new light onto the aetiology of biliary atresia. Isolation of a previously unidentified isoflavonoid, biliatresone, from *Dysphania* plants has been found to cause damage to the extrahepatic biliary system in zebrafish larvae. These larvae develop a phenotype similar to that of human biliary atresia.

Biliary atresia, a progressive disease of extrahepatic bile ducts (EHBD), although rare, is the primary indication for liver transplantation in the paediatric population. Therapeutic options are limited; the mainstays of treatment are surgical, with no targeted medical therapies. The lack of treatment options is partly attributable to our incomplete understanding of the aetiology and pathogenesis of biliary atresia. So far, evidence implicates both genetic and environmental causes of disease.

Outbreaks of biliary atresia in lambs and calves in NSW, Australia, have occurred several times, always during periods of drought. "It was strongly suspected that ingestion of unusual plants [made available during droughts] by pregnant cows and ewes was responsible," explains Wells. Stephen Whittaker, co-author of the paper, suspected that *Dysphania* species were the culprit and so samples were imported into the US for analysis.

"We recognized the potential of the zebrafish as a screening assay to identify active compounds in the plants," says Pack. Initially, zebrafish larvae at 5 days postfertilization (dpf) were exposed to three crude chemical fractions from the plants for 72 h. Fluorescent staining revealed that one fraction caused a substantially reduced fluorescent signal from the gallbladder and intestines.

After many more rounds of fractionation, biliatresone was isolated and identified as the active toxin. Morphological disruption of the extrahepatic biliary system was found to be dose-dependent, with more disruption in the high-dose groups than the low-dose groups. In the high-dose groups, EHBD were difficult to distinguish and the gallbladder was extremely abnormal. The ability of biliatresone to induce a biliary atresia phenotype was also time-dependent. Biliatresone had no effect when administered at early stages of development (6 h and 24 h after fertilization); however, at later time points (8 dpf and 13 dpf), a sensitization to biliatresone occurred. In all treated zebrafish larvae, the intrahepatic bile ducts were unaffected, which suggests biliatresone has specificity for the extrahepatic biliary system.



Mouse cholangiocyte spheroid cultures treated with vehicle (left; ×250 magnification) and biliatresone (right; ×120 magnification) and stained with F-actin (red), integrin-β1 (green) and DAPI (blue). Image courtesy of Orith Waisbourd-Zinman.

The authors hypothesized that the toxicity of biliatresone was a result of its secretion and concentration in bile. First, larvae were treated with a Notch inhibitor that blocks formation of intrahepatic bile ducts, which was expected to ameliorate biliatresone toxicity. Gallbladder morphology was relatively normal whether or not these larvae were treated with biliatresone, which supports the hypothesis. Second, a genetic mutant, called *ductbend,* lacking in intrahepatic bile ducts (but with a gallbladder and EHBD) was treated with biliatresone. Surprisingly, the phenotype was more severe in treated than untreated *ductbend* mutants and also compared with wild-type larvae, which suggests a synergistic effect exists between the mutation(s) and the toxin. Further investigation by whole-exome sequencing revealed conserved areas of homology between the *ductbend* locus and a region close to *ductbend,* to two human biliary atresia susceptibility loci (10q25.1 and 16p13.3).

Disrupted integrity of the EHBD epithelium and fewer cholangiocyte primary cilia are characteristics of human biliary atresia and some mouse models. A loss of monolayer integrity and disrupted apical polarity in mouse cholangiocyte spheriods was observed with biliatresone treatment. In addition, biliatresone caused a reduction in the number of primary cilia of neonatal mouse cholangiocytes *in vitro.*

The authors argue that these results suggest a common pathology between their *in vivo* and *in vitro* biliatresone models and human disease. "We have a new animal model of biliary atresia and hope to soon have an *in vivo* rodent model," says Wells. "We will define the toxin's mechanism of action and will use the information to look for environmental triggers of [human] biliary atresia," concludes Pack.

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Original article Lorent, K. *et al.* Identification of a plant isoflavonoid that causes biliary atresia. *Sci. Transl. Med.* **7**, 286ra67 (2015)