

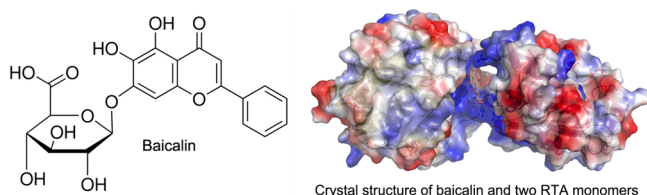
■ QUORUM-SENSING *SALMONELLA* ENABLE DENSITY-DEPENDENT PROTEIN EXPRESSION EXCLUSIVELY IN TUMORS

Tumors produce bacterial chemoattractants which lead to high levels of *Salmonella* colonization. Since *Salmonella* penetrates tumor tissue, it can be utilized as an expression vector to activate anticancer drugs. However, as there are low levels of *Salmonella* in healthy tissues, constitutive protein expression could detrimentally lead to systemic dosing. Since 10,000-fold more *Salmonella* are present in tumors than in other organs, it would be advantageous if bacterial density could direct the location and timing of drug production.

Quorum-sensing (QS) bacteria use population density to control protein expression. Neil S. Forbes led a team which integrated a QS gene expression switch with a GFP- fluorescence reporter into an attenuated *Salmonella* strain ((2015) *Proc. Natl. Acad. Sci. U.S.A.*, 112, 3457–3462). They hypothesized that QS-controlled *Salmonella* protein expression would be induced only in the presence of high bacterial density, which should be limited to cancerous tissues. The researchers used *in vitro* and *in vivo* studies and found that while both constitutive and QS *Salmonella* preferentially accumulated in tumor tissue as expected, the former bacteria expressed GFP in a density-independent fashion, while the latter induced GFP expression only at high densities. Moreover, equally dense colonies that differed in the distances between themselves and their neighbors had different GFP expression patterns, indicating that QS activation is determined by high bacterial density and close spatial distribution. The authors were unable to detect GFP expression in healthy liver tissue after QS *Salmonella* administration but observed GFP expression in tumor tissue as late as 24 days postinjection. The low toxicity of this approach permits systemic administration of therapeutic proteins which could target established tumors and undiagnosed metastases.

Abigail Druck Shudofsky

■ BAICALIN INHIBITS RICIN TOXICITY BY INDUCING OLIGOMER FORMATION



Adapted from Dong et al. (2015) *J. Biol. Chem.*, DOI: 10.1074/jbc.M114.632828. Copyright 2015 American Society for Biochemistry and Molecular Biology.

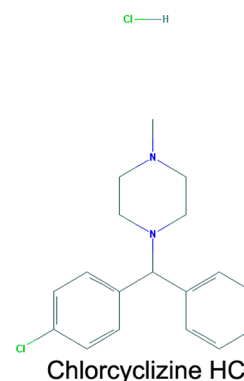
Ricin is a highly poisonous protein that can be extracted from castor beans. Its toxicity lies in its ability to inactivate ribosomes, inhibiting protein synthesis and causing eukaryotic cell death. Xuming Deng, Xuemei Li, and their teams discovered that baicalin, a flavonoid compound extracted from the Chinese herb Huang-chin (*Scutellaria baicalensis*), can inhibit ricin toxicity ((2015) *J. Biol. Chem.*, DOI: 10.1074/jbc.M114.632828). *In vitro* studies showed that baicalin pretreatment prevented HeLa cells from ricin-induced death with negligible effects on viability, as it did when it was applied to the cells in combination with the toxin. *In vivo* mouse studies showed that baicalin rescued 50% of animals exposed to lethal doses of ricin when given 6 h postexposure and every 6 h thereafter for the duration of the experiment. Baicalin also alleviated other pathologies associated with ricin poisoning, such as decreased blood glucose levels and elevated cytokine levels.

Ricin is made up of two subunits; a catalytic A chain (RTA) that is an RNA N-glycosidase and a B chain that is important for cell entry. As their data indicated that baicalin directly targets ricin, the authors solved the crystal structure of baicalin complexed with RTA, illuminating its unique mechanism of action. Baicalin does not change the structure of RTA upon binding or occupy its active site; rather, the inhibitor uses

extensive hydrogen bonding to position itself at a novel binding site in the positively charged interface of two RTA molecules. This causes RTA oligomerization and blocks substrate access to the active site on the RTA monomer. The resulting loss of N-glycosidase catalytic activity is significant and prevents translation inhibition, leading to lower ricin toxicity.

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■ ALLERGY DRUG IS A POTENT INHIBITOR OF HCV INFECTION



National Center for Biotechnology Information. PubChem Compound Database; CID = 62413, <http://pubchem.ncbi.nlm.nih.gov/compound/62413> (accessed May 15, 2015).

Hundreds of millions of people globally are chronically infected with hepatitis C virus (HCV). HCV infection is usually asymptomatic, but left untreated, it can lead to inflammation and the development of liver diseases. HCV antivirals are available, but they are expensive, prone to genetic drug-resistance, and may have side-effects and interact with other drugs. As polypharmacology is common, T. Jake Liang led a

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team screening existing FDA-approved drugs for use against HCV infection ((2015) *Sci. Transl. Med.*, 282, 282ra49). Using a cell-based high-throughput screen, they identified chlorcyclizine HCl (CCZ), an over-the-counter allergy drug, as having high anti-HCV activity.

CCZ is an affordable first-generation H₁-antihistamine with an established clinical safety profile, though its anti-HCV activity is likely unrelated to its interaction with the histamine receptor. The researchers found that *in vitro* and *in vivo*, CCZ inhibits intracellular viral RNA in infected hepatocytes in a pan-genotype, dose-dependent manner. The drug had little or no cytotoxicity after continuous treatment for 21 days. When CCZ was used in combination with other established anti-HCV drugs, there was a synergistic inhibitory effect, supporting its use in combination therapy and suggesting that it targets a different mechanism of the viral lifecycle than the other included drugs from various classes. The scientists determined that CCZ inhibits an early stage, late-entry step of HCV infection before RNA replication without directly affecting HCV entry factor expression levels or cellular distribution. Moreover, their research suggests that CCZ does not promote the emergence of drug-resistant viruses. Despite its use as an allergy drug, CCZ is specific to HCV and displays minimal inhibitory activity against 13 other viruses. Further testing of CCZ is needed to determine its efficacy against HCV in humans. In addition to its high potency and selectivity, CCZ has a simple chemical structure that can be further optimized for anti-HCV drug development.

Abigail Druck Shudofsky

■ β -CELL RESPONSE TO NITRIC OXIDE IS DETERMINED BY THE SITE OF SUPEROXIDE FORMATION

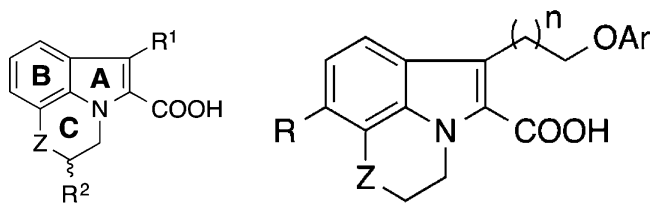
Inflammatory cytokines can damage insulin-producing pancreatic β -cells, impairing their function and resulting in type 1 diabetes. This harm is mediated by inducible nitric oxide synthase (iNOS), which produces high levels of nitric oxide (NO) in β -cells. NO inhibits mitochondrial oxidative metabolism, causing a decrease in ATP synthesis which leads to impairment in insulin release. When NO reacts with superoxide, a reactive oxygen species (ROS), it forms peroxynitrite, a reactive nitrogen species (RNS) that might be the mediator of cytokine-induced β -cell damage. John A. Corbett and his group looked at the sensitivity of β -cells to ROS and RNS and found that both forms of reactive species can activate signaling cascades which modify β -cell function and viability but that the cellular response is dependent on the type, form, and concentration of the reactive species ((2015) *J. Biol. Chem.*, 290, 7952–7960).

NO diffuses through cell membranes and generates peroxynitrite when it encounters superoxide, typically produced exogenously by macrophages. However, while β -cells do not characteristically generate superoxide, they can be compelled to do so. Interestingly, the researchers found that the response of an individual β -cell to NO is determined by the location of superoxide generation. Extracellularly produced superoxide renders β -cells vulnerable to NO-mediated effects. However, intracellularly produced superoxide scavenges and binds cellular NO, preventing its inhibitory effects on β -cell oxidative metabolism. Superoxide produced within cells also prevents NO-induced ATP depletion, while exogenous superoxide leads to an NO-dependent decrease in intracellular ATP levels. Similarly, only when NO is combined with intracellularly produced superoxide can its stimulatory effects on NO-dependent gene expression be attenuated. While comparable levels of peroxynitrite are

produced regardless of the site of superoxide generation, only when the ROS is generated internally does it protect β -cells from NO-mediated cellular toxicity.

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■ FRAGMENT-BASED METHODS AND STRUCTURE-BASED DESIGN HELP DETERMINE TRICYCLIC INDOLE INHIBITORS OF MCL-1



Tricyclic Indole 2-carboxylic Acids

Merged Tricyclic Indole Derivatives

Adapted from Burke et al. (2015) *J. Med. Chem.*, 58, 3794–3805. Copyright 2015 American Chemical Society.

Apoptosis is positively and negatively regulated by stress-responsive proteins. The antiapoptotic protein myeloid cell leukemia-1 (Mcl-1) is overexpressed in numerous human cancers and is associated with poor survival, as it allows cancer cells to evade apoptosis and is implicated in chemotherapy resistance. There is no available therapeutic that selectively targets Mcl-1, though compounds that successfully neutralize it can potentially restore successful positive regulation of apoptosis and enhance responses to cancer treatment. Stephen W. Fesik led a team to identify small molecule inhibitors that bind Mcl-1 ((2015) *J. Med. Chem.*, 58, 3794–3805). They had previously discovered a tricyclic indole 2-carboxylic acid core fragment (figure, left) using an NMR-based screen. The team now performed structural studies and investigated structure–activity relationships to optimize derivatives of this hit.

The authors determined that C-ring modifications can enhance Mcl-1 interactions and improve binding affinity, as can substituting methyl groups at R¹ or R² positions. A model structure predicted that R¹ can ideally extend deep into the lower part of a hydrophobic pocket in Mcl-1, which impacts binding affinity and likely also determines Mcl-1 selectivity. With this knowledge, the authors merged suitable molecules with tricyclic indoles to synthesize a new series of inhibitors with enhanced Mcl-1 binding affinities (figure, right). They found that the position of the pocket anchor group was integral to optimal binding. The group also found that Mcl-1 binding affinity was significantly increased when four-atom linkers were used; when 6-membered C-ring moieties with S- or O-compositions were used; and when 6-Cl-tricyclic indoles were present in the B-ring. The researchers discovered highly potent and selective Mcl-1 inhibitors with single digit nanomolar binding affinity.

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