Nature Reviews Chemistry | https://doi.org/10.1038/s41570-018-0017-7 | Published online 19 June 2018

DRUG METABOLISM

Deciphering digoxin deactivation

Credit: Robert Garrigus/Alamy Stock Photo

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Digoxin is a widely used heart drug, but metabolism by bacteria in the human gut leads to variable efficacy. The enzyme responsible has now been identified and characterized.

Microbes that live in the human gut can play a vital role in our metabolism of drugs. Now, writing in *eLife*, Emily Balskus, Peter Turnbaugh and co-workers have identified and characterized the bacterial enzyme that causes inactivation of digoxin — a widely used heart medication.

A glycoside originally isolated from foxgloves, digoxin can be toxic but at low doses is widely used to treat a variety of heart conditions — so much so that it is on the WHO list of essential medicines. "It was known since the 1960s that certain patients taking digoxin needed to take much higher doses of this drug than others to see efficacy," says Balskus. "Eventually, this was ascribed to conversion of digoxin into inactive dihydrodigoxin by the bacterium *Eggerthalla lenta*." But this alone did not explain the problem. Some patients known to host this bacterium in their guts did not have problems with drug inactivation. So Balskus, Turnbaugh and their groups set out to identify and characterize the enzymes involved.

The team began their investigation by collecting 25 strains of *E. lenta* (and the closely related *Coriobacteriia*) and, using liquid chromatography–tandem mass spectrometry, identified 7 that were capable of digoxin inactivation. Sequencing these strains led to the identification of a common gene cluster encoding eight different proteins. Three of these proteins (Cgr1, Cgr2 and Cac4) showed sequence homology to reductase enzymes. In addition to studying DNA sequences, the team had previously reported RNA sequencing studies showing that genes encoding Cgr1 and Cgr2 are upregulated in response to digoxin. The presence of proteins similar to Cgr1 in strains of *E. lenta* that do not metabolize digoxin narrowed the field of suspects to a single enzyme, Cgr2. Expression of this enzyme in a different bacterial host showed that it is Cgr2 that deactivates digoxin.

Having identified the enzyme responsible, the team then characterized its activity. The presence of 16 cysteine residues in Cgr2, as well as its increased stability in the presence of iron and sulfide, strongly hinted that the enzyme contained an iron–sulfur cluster. Indeed, UV–visible experiments confirmed this with electron paramagnetic resonance (EPR) spectra featuring signals characteristic of a 4Fe–4S cluster in particular. "This was unexpected," explains Balskus, "as the enzyme doesn't contain any of the previously known sequences for Fe–S cluster assembly."

The researchers proceeded to screen the activity of Cgr2 towards 28 small molecules similar in structure to digoxin. Surprisingly, the enzyme was found to be active only for the reduction of digoxin and some closely related plant toxins (cardenolides). "The specificity suggests that these microbes may have evolved to protect humans from the toxic effects of these plants," says Balskus.

An outstanding question is whether this information might be used to the benefit of patients who need to take digoxin. Physicians may eventually be able to predict patient response to the drug or could even attempt to design a therapy that can avoid this metabolism.

"We remain fundamentally interested in both the structure of the enzyme and its Fe–S cofactor," says Balskus. The presence of this redox cofactor makes sense given that the enzyme must deliver $2H^+$ and $2e^-$ to deactivate digoxin by hydrogenating its 2-furanone group. The team are also keen to learn more about how and where this activity arose and why it is of benefit to the bacteria. "Did it evolve in the human gut, at a time when we more routinely ingested low levels of toxins? Or did it evolve in the gut of insects that usually consume the plants that produce the cardenolides," wonders Balskus.

The work also opens the way for studies on the metabolism of other important xenobiotic compounds. More than 50% of the genes encoded by the gut microbiome have unknown function. "Having studied Cgr2 and its metabolism of digoxin, we have subsequently identified many enzymes produced by human gut microbes that are likely to perform similar reductive reactions, but we don't currently know the substrates they act on," says Balskus.

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ORIGINAL ARTICLE Koppel, N. et al. Discovery and characterization of a prevalent human gut bacterial enzyme sufficient for the inactivation of a family of plant toxins. *eLife* **7**, e33953 (2018)