In Search of an Armor-Busting Antibiotic

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SPEAKERS
Maggie Fox and Skyler Cochrane

Maggie Fox 00:00
Hello and welcome to One World, One Health with the latest ideas to improve the health of our planet and its people. I'm Maggie Fox. All of us here on planet Earth are facing problems, such as pollution, climate change, and infectious diseases (old and new). Animal health, human health, plant health, and the climate; they're all linked. This podcast is brought to you by the One Health Trust with bite-sized insights into ways to help.

The world desperately needs new antibiotics, but bacteria are outwitting scientists and drug developers at every turn. Germs live in their own competitive world and they've evolved mechanisms to keep rivals and enemies at bay. This makes them naturally resistant to the drugs we, humans, try to develop to fight them. On top of that, our own use of antibiotics leads to the inevitable rise of bacteria that can survive their effects. Labs around the world are working to develop new and better antibiotics, but it's a hard fight. Most new drugs fail in development. And nowhere is this more true than with antibiotics.

In this episode of One World, One Health, we're chatting with Dr. Skyler Cochrane, a research scholar at Duke University who is part of a team working on promising new antibiotics, and one that seems to work safely against a range of bacteria. Tests in mice suggest it can kill bugs, such as Staphylococcus aureus, and Klebsiella, which are among the biggest gram-negative killers. We're hoping to learn a bit more about how difficult it is to develop new antibiotics and what her team has found in the lab. Skylar, thanks so much for joining us.

Skyler Cochrane 01:36
Thanks so much for having me. I'm really excited to be with you guys today.

Maggie Fox 01:39
Can we go back to basics and talk about gram-negative versus gram-positive bacteria? What's the difference?

Skyler Cochrane 01:48

So, starting with just the name itself, gram-positive versus gram-negative. We're like, what does this mean? There is a very special dye, it's a purple color, and it's called a gram stain. And what happens is when you coat bacteria in this stain, or submerge them in a solution, you'll either see the bacteria absorb the stain and turn purple, or you'll see that they don't have any color change at all. And that's where gram-positive comes from. It means it absorbs the gram stain. Gram-negative does not absorb it. And the main reason for this comes down to the actual composition of the cells themselves. So, what makes gram-negative bacteria not absorb the stain and in turn, makes them more difficult for our medicines is the fact that gram-negative bacteria have a double membrane in their cell envelope. But typically, when we think of a cell wall, that's referred to as peptidoglycan.

And gram-positive bacteria have a thick layer of that along with an inner membrane. But with gram-negative bacteria, that sort of cell wall component is thinner. And then you have another outer membrane sandwiching it, creating this like full cell envelope that's much more difficult for drugs or foreign substances to penetrate.

Maggie Fox 02:58
And that's why they're harder to kill. The drug can't get in.

Skyler Cochrane 03:02
Gram-negative bacteria are basically wearing an extra suit of armor in comparison to the gram-positive counterparts.

Maggie Fox 03:09
Gram-negative bacteria, as you say, are sometimes naturally resistant to antibiotics just because of their structure and for other reasons as well. But they can also acquire resistance. Can we talk a little bit about the difference between evolved resistance and acquired resistance?

Skyler Cochrane 03:26
Absolutely. So, starting on what you were just saying, we do have like those two basic, there's intrinsic resistance, which means just the composition of the bacteria itself prevents the drug from working for whatever reason. And then acquired resistance comes in two different forms. And this can either be through a genetic change. So, for instance, the bacteria will evolve some small change somewhere in their DNA that allows them to overcome the bacteria.

So, let's say you have a protein target and your drug binds to it usually. The bacteria can sometimes evolve a slight change in the composition of that protein, where suddenly there's something occupying that space or blocking the penetration of the drug from only a single amino acid mutation. And that's evolved resistance.

Now acquired resistance is kind of fascinating. In that, bacteria can actually transfer their own genes to each other. So, let's say one bacteria develops a resistance mechanism, it evolves that special little change that we made. It can basically send that little packet of DNA over to other bacteria in the area that can then absorb it and now, they can implement that resistance mechanism as well. It becomes a part of their new DNA.

Maggie Fox 04:34
And, to turn to where antibiotics come from, the the first true antibiotic penicillin came from nature. In that case, it was mold and other antibiotics, such as cephalosporins, came from fungus as well. Bacteria pump out their own antibiotics, but you can also design them in the lab and build them from the ground up. Is that what you're doing?

Skyler Cochrane 04:56
Yes, absolutely. That is one of the main things that we work on right now. While a lot of current drugs on the market do come from those sort of natural products that we are discussing, it's kind of hard to identify those sort of new products and identify new ones from nature. So, what we do is there's so many libraries of millions and millions of compounds and drugs that scientists have isolated and have collected over the years.

And whenever we have a new target that we want to look at, we can do what's called High Throughput Screening, where you can screen libraries of all of those millions of drugs against your target in a very fast method. And from those, you can get a couple of ones that pop up that have at least some of that. And then, we can look at that base structure, see how it actually works in the enzyme, create a model of it, and then start to make changes using a rational thing. So, if we look and we see our drug occupies this one small pocket, but there's all this other room around it, we can use our chemical knowledge to think of extra groups that we can add ------. We can extend it to create new little interactions.

One of the ways that I like to think about proteins is like a really, really complicated 3D lock. And our drug design is about crafting the perfect key—starting from one little piece at a time. And the way that we do this is this technique of development called Structure-Activity Relationship, or SAR, which is my favorite method for drug development. It's got a very logical progression to it. So, where it starts is, you know, we have this compound that we think could work. And what we'll do is we'll test it against our purified target on its own, then we'll test it against the bacteria itself.

So first, we see does it work in our target ------. Next, we'll test and see can it penetrate the bacteria to get to its target. Though, (can it be) we'll have efficacy there. And then when we see that our drug is effective in both, we'll do this technique called X-ray crystallography. And basically, what this is, when we think of things like crystals, we think of (like) diamonds and stones and all of those things. But crystals can actually form out of so many different materials, including compounds and proteins (can form crystals).

So, what I do is I will form crystals of my protein with the drug present. And these crystals are microscopic. They're grown in a two microliter drop. We have to scoop them under a microscope. I have this little tiny ------. So, my friends refer to me as a crystal cowboy. It's kind of fun. But once we harvest these crystals, we can ship them off to a facility where they will shoot X-ray beams at the crystal. When crystals form, it's basically a perfect lattice structure of whatever your material is. So, it will form in this perfect reflective state.

So, when an X-ray beam hits it, it's going to diffract those beams at different angles and unique ways depending on what your core structure is. So, basically, that beam will hit it and you'll have a whole room of diffracted X-ray beams with sensors that can pick that up and then reverse engineer an atomic level 3D model of a protein. And you can literally see the presence of your drug in it, in this atomic density.

So, from that, we're able to create these beautiful crystal ribbon diagrams, where we can see where the drug is present, where it's interacting, and then use that to make new designs. We send that back to our ------ with our idea; they'll help us synthesize the new drug. And we'll start the process all over again. And then once we feel like we have good enough properties, we will advance that to further trials. In our case, we go to mouse models.

Maggie Fox 08:14
So, you're using over and over again this word target, and you have one. Can you tell us a little bit about the target that you're working on?

Skyler Cochrane 08:21
Yes. So, we are targeting a protein specifically called LpxC. This protein is part of a biosynthetic pathway for a very special component in gram negative bacteria. So, I was telling you before how gram-negative have a special double membrane and an extra layer of shielding. When we look at that outer membrane itself, the very outermost component of it is formed by something called lipid A. So, lipid A binds together on the very, very outer surface.

You can almost think of it like the epidermis of the bacteria (that outer layer), that if you could touch it, (it) would be what you interact with. Basically, lipid A is the first line of defense that gram negative bacteria have to drugs. So, the synthetic pathway how the bacteria creates lipid A is a seven-step pathway of different. So, (we'll start with) it'll start with a base component. And it will go through each of these proteins.

Basically, they're little machines. When you have something, it goes into the machine, (and) it comes out slightly different or ------. And ------ the pathway itself is a conveyor belt of these different machines. And at the end, you get the final lipid A component that goes out, forms this layer. Our target LpxC is the second step in this pathway. It's been found that if you can inhibit the production of lipid A, you can cause cell death and many gram-negative bacteria because it will essentially destabilize that outer membrane and contribute to the eventual falling apart of the bacteria.

So, with our target, if we can stop it from working, we can clog up that conveyor belt pathway and the bacteria will eventually die.

Maggie Fox 09:54
And it looks like this compound safely and effectively can kill a range of bacteria. Can you tell us a little bit how it does that and the safety factor as well?

Skyler Cochrane 10:04

Not just a few bacteria, we tested our drug against over 285 different strains, including both resistant and non-resistant strains that have already been identified. So, we found that largely, it is not affected by known resistance mechanisms, which is very exciting. And then, we did a bunch of different safety and efficacy testing of this drug. So, to begin with, usually, once we have a drug that we want to take to some sort of live model testing, we'll start with different toxicity studies.

So, for instance, we will put mammalian human cells and put them in the presence of the drug, (and) make sure it's not toxic to human cells to begin with. Next, we'll start doing studies in mice and rats. So, we did a seven-day toxicology study in rats, where we gave them high doses of the drug just to see would it affect them, would they show any negative side effects. And we found that it was completely safe there. We next moved on to mouse model. So, we tested two different strains of E. coli that have certain drug resistances already.

And what we found was when we dose them for five days, twice a day, we were able to 100% clear the infection from both bacteria in these mice. We repeated the studies using orally administrated or orally given drugs instead of our usual injections. And we found that the drug was just as effective when orally dosed, instead of injected. And why this is important is it's fine when you're in a hospital to have to get an injection of a medicine, right. But no one wants to take needles home and have to give themselves shots at home when they're already feeling sick and having an infection.

So, when it comes to something that can be broadly given to the general population—a drug—you always want it to ideally be orally available, which means you can take it as a pill, and it can be absorbed through your stomach. So, we were really excited to see that our drug worked through both different ways—that it could be absorbed by your body when given that way.

Maggie Fox 11:52
So, let's just be clear. You have not tested this in people yet.

Skyler Cochrane 11:56
We would ideally like to eventually move to human studies. That is the goal of this work. It still might be a little while before we reach that step, because there's a lot of other processes involved before that, including formulation, testing, and other things. Because you don't want to give it to humans until you feel really, really good about it.

So, you got to keep in mind, these are human lives that we're doing. And medicine is designed to help heal. And we want to make sure that before it goes into a person that we feel the most confident about it that we can.

But, so far from all of our studies, this is the most promising LpxC inhibitor to be produced to reach this sort of stage. There only have been two other inhibitors of our enzyme in history that have made it to clinical trials. But both of them got disqualified early on because they were found to induce cardiovascular toxicity. So, they were causing issues with heart and causing heart damage.

But one thing that we showed in our study that was exciting is we actually did cardiovascular studies because of this known effect. And we found that we had no cardiovascular toxicity and no changes in the heart health with administration of our drugs. So, that means we are the first inhibitor to not induce this toxicity, and to have this high level of efficacy. So, we do hope to eventually progress this to clinical trials. And we hope that we have overcome this obstacle that has been in the way of this target in the past.

Maggie Fox 13:18
But a lot more can go wrong. We know drug testing is hard and most antibiotics fail in testing. What do you see that might go wrong as you go forward?

Skyler Cochrane 13:27
There's always so much that can go wrong in science. So, there's only so much that we can test ahead of time that we think of. So, for instance, like I was mentioning with those two previous inhibitors, they didn't know there would be cardiovascular toxicity before they went to clinical trials. It was something that they didn't know until they got there. And in the same way, we can't predict what side effects or off target effects we might have further down the way.

That's only something that time can tell. But we do have the promising features from our preliminary studies that we are very hopeful (for). And one of the good things for us is since we're targeting something that is exclusive to gram negative bacteria, LpxC doesn't exist in humans. It doesn't exist in a lot of the places we would be concerned (about). So, as long as our drug, if we can make it (that perfect key that only fit that lock), we're very hopeful that it won't have any off-target effects.

Antibiotics are grouped into things called classes and a class of antibiotics are defined by core structure. They'll all share this very similar center point with just different variations off of them. So, if you think penicillin, amoxicillin, ampicillin, that "cillin" is the reference to the class itself. And they all contain this very specific ------ with variations. So, we haven't had a new class of antibiotics discovered since the 80s (so, in over 40 years). And where that becomes a problem is usually when bacteria develop resistance, they develop class-based resistance.

So, mechanisms that are designed to be resistant to that base feature. So, they'll work against multiple in that area. So, as we, (are) in hospitals, and we have infections and resistant ones, we keep treating them, but the longer we treat them, the more class resistances these bacteria can build until sometimes (when) we don't have anything that can work anymore.

Working on these sorts of things, developing this LpxC inhibitor would be a new class of antibiotics should they make it to market or get past clinical trials. So, it would be the first new class of antibiotics to be approved in over 40 years if it makes it that far down the line.

Maggie Fox 15:28
Skyler, thank you.

Skyler Cochrane 15:30

Thank you so much for having me. This has been a joy and a pleasure. I love getting to talk about my research and share more information about science and medicine.

Maggie Fox 15:40
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