New method to overcome multiple drug resistant diseases developed by Stanford researchers

August 18 2008

Many drugs once considered Charles Atlases of the pharmaceutical realm have been reduced to the therapeutic equivalent of 97-pound weaklings as the diseases they once dispatched with ease have developed resistance to them.

The problem is well documented for antibiotics, although not confined to them. Chemotherapy drugs that were once highly effective when first used against a particular cancer now are often rendered near powerless when a patient's cancer resurges.

Even more devastating, when an organism develops resistance to one drug, it often becomes resistant to other drugs (known as multi-drug resistance), rendering not just one medication but a whole class of therapeutics useless against it.

But researchers at Stanford University have developed a method to get around one of the most common forms of resistance, thereby opening up some if not many resistant diseases to the reinvigorated fury of the medications that once laid them low. To do it, they took a tip from nature.

"Nature has developed all of this firepower for getting things into cells, and one of the ways is to create entities that are arginine-rich," said Paul Wender, the Bergstrom Professor of Chemistry at Stanford University.

Arginine is an amino acid, the building block of proteins, and as such is found in virtually every cell in the human body, as well as other mammalian bodies.

Using such a common transporter to ferry a potent medication inside a resistant cell is a bit like recruiting your grandmother to cart a load of switchblade knives through customs. Indeed, Wender said, "Arginine-rich sequences appear to figure in the mechanisms by which many pathogens invade cells." Wender's team used a necklace of eight arginine molecules to surround the medication they worked with.

Wender and his colleagues figured out that a particular molecular subunit within arginine, called a guanidinium group, was what nature actually exploits to get foreign substances through cell membranes. Working with Taxol(r), a widely used chemotherapeutic agent, they attached a series of arginines with their associated guanidinium groups and tried it out against Taxol-resistant ovarian cancer cells implanted in mice. It worked.

"It's an exciting result to be able to take a drug known to work against cancer, but stymied by resistant cells, and restore it to effectiveness using an arginine transporter," Wender said. "This bodes well for use with other drugs that succumb to resistance."

A paper describing the work is scheduled to be published next week in the online Early Edition of the *Proceedings of the National Academy of Sciences*. Wender's group collaborated with that of Chris Contag, a professor of pediatrics and of microbiology and immunology at Stanford's School of Medicine, who is a co-author on the paper.

"Overcoming Taxol resistance is big. It's huge," said Nelson Teng, professor of obstetrics and gynecology at the Medical School. "In essence, the technology can be used to overcome one of the most

challenging types of problems of drug resistance."

The type of drug resistance that Wender's work has overcome develops when pumps located in the membrane that encloses a cell become sensitized to a medication. It is one of the most common ways in which resistance manifests. The pumps, which normally capture and eject foreign material from a cell, are produced at higher levels in certain resistant cells and, because of their increased number, become more effective at tossing the drug molecules out.

"It is kind of like a bouncer," Wender said. "If you're not recognized as being part of the club, then you're kicked out." Resistant cells also create a lot more of the pumps than a normal cell would have.

Some researchers have tried dealing with this situation by adding another molecule to the mix to inhibit the pump, keeping it busy so the medication can slip in while the pumps are occupied with the decoy molecule. But if any of the molecules make their way into healthy cells, they can gum up the proper functioning of the pumps in those cells, too, adding to the litany of undesirable side effects that generally accompany chemotherapy.

Wender's group decided to see if they could take drugs to which diseases had become resistant and, by combining them with what they call "molecular transporters," get them in around the pump.

"If we think of the pump as being a bouncer for the cellular club, then effectively what we're doing is disguising one of these therapeutic agents to get it in through the back door or the side door," Wender said. "We're not even going to deal with the bouncer."

Therein lies what may be the greatest value of the work. The basic approach of bonding a medication to an arginine-rich transporter to slip

it past the cellular sentries could, in theory, be used to get any of a host of medications into any cell that has developed the type of resistance involving increased numbers of export pumps.

"This could potentially be used with any drug which is effective but has a delivery problem," Teng said. "Not just Taxol."

That could include medications for diseases caused by antibiotic resistant bacteria, such as multi-drug resistant tuberculosis, or by drug resistant parasites such as malaria, as well as other types of cancer.

The arginine transporter manages to avoid ejection by slipping through the membrane of the cell in between the pumps. The key is the ability of arginine to form weak, temporary bonds with some of the molecules that reside in the membrane.

"As the transporter, with all these arginine guanidinium groups, approaches the cell, it basically does a handshake using hydrogen bonds with cell surface constituents that are in the membrane," Wender said. "In essence, it changes its physical properties by shaking hands with all these cell membrane components."

That change in physical properties effectively cloaks the arginine-Taxol complex, allowing it to slip past the sentries and into the cell. As it passes into the cell, the weak bonds it formed with the membrane components break and the transporter, with its therapeutic load, is free to roam inside the cell.

But after getting into the cell, the arginine-Taxol complex still has to break apart for the Taxol to do its job against the cancer cell. Wender's group achieved this by taking advantage of the presence of a molecule called glutathione, which is generally abundant inside cells and which in cancer cells tends to be present in higher levels than usual.

Glutathione is predisposed to attacking sulphur-sulphur bonds, so that is the bond the researchers used to hold the arginine and Taxol together. Once the arginine-Taxol complex is inside the cell, the glutathione can get to work hacking away at the sulphur bonds, and in the process, unwittingly release the compound that will spell its doom.

Because glutathione is relatively scarce outside of cells, the arginine transporter is effectively inert in that environment, so there are no side effects from having the arginine-Taxol complex moving through the patient's body. This is in stark contrast to the present situation, as many patients are extremely sensitive to the molecular vehicle that is currently used to administer ferried Taxol to the cancer cells.

The researchers achieved another breakthrough by tinkering with the form of the arginine used in their transporter. By altering certain aspects of the arginine, the researchers were able to control the rate at which glutathione slices and dices the arginine-Taxol complex.

This gives them an unprecedented ability to regulate the amount of medication that is active inside the patient at any point in time. To date, doctors have had to be content with injecting as high a dose of medication as patients can tolerate and then waiting as the effective amount in the patients slowly dwindled until they could safely inject more. This approach results in a repeated pattern of rapid spikes in the amount of medication in the system, followed by slow declines until the next spike. Ideally, doctors would like the patient to be continually experiencing the maximum tolerable dosage to keep the pressure on the cancer cells, killing them off as quickly and as thoroughly as possible. The arginine transporter makes this possible.

Ovarian cancer was chosen as the subject cancer for this study in part because it commonly develops resistance to Taxol, but also because of a low long-term success rate in treating it. The American Cancer Society

estimates that in the United States alone there will be 21,650 diagnoses of ovarian cancer this year and 15,500 deaths from it.

"Ovarian cancer has a drug [Taxol] that works pretty well in the beginning. Seventy or eighty percent of the patients have a response," Teng said. "But it fails at the end because drug resistance develops."

Further studies need to be done to demonstrate the safety of arginine transporters before they can be used in this application in humans, Wender and Teng said. But the researchers already have positive safety data from tests of arginine-transporter technology in another application, one that does not involve drug resistance, so they are optimistic. The discovery of effective arginine transporters could be the key to treating ovarian cancer, as well as other diseases that develop drug resistance, more effectively.

Source: Stanford University