Slowing Down the Spread of Antibiotic Resistance with Gene Transfer Disabling Drugs

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Bio 25L Section 23

February 23, 2000

Gene transfer disabling (GTD) drugs are a proposed class of drugs that will greatly enhance the effectiveness of current antibiotics. GTDs may range in size from small molecules, proteins, other macromolecules, or even short strands of DNA or RNA, depending on what the drug is designed to bind to and how the drug will be introduced into a bacterial cell. Gene transfer disabling drugs do exactly what their name describes—they slow down the spread of antibiotic resistance by blocking the passage of resistance genes between bacteria. If used in conjunction with antibiotics, GTDs have the potential to greatly reduce the spread of antibiotic resistance, giving researchers more time to develop new antibiotics before current ones are rendered ineffective.

Antibiotic resistance is the result of the selective pressure imposed on a bacterial population by antibiotic treatment. When resistant individuals outnumber and reproduce more than non-resistant individuals, those resistance genes then become passed to more bacteria, thereby spreading antibiotic resistance. The first obvious method of gene transfer is *cell division*, where a cell makes two identical copies of itself. However, the method of gene transfer most responsible for antibiotic resistance is *conjugation*, during which tiny circular strands of DNA called *plasmids* cross a tube connecting two bacteria. These plasmids often contain resistance genes, and through this process, resistance is passed on.

If researchers could develop drugs that blocked the flow of resistance genes between bacteria, this would significantly help to decrease the spread of antibiotic resistance. This is why gene transfer disabling drugs are important. At the University of Arizona, a group of researchers have taken the first step toward developing conjugation-inhibiting GTD drugs. Specifically, the goal of this research group is to "nullify bacterial resistance to antibiotics by inhibiting the necessary genetic exchange of material via conjugation" (Escalante *et al.*, 1995). Here are some alarming statistics from one of their reports:

> *-Every disease causing bacterium now has strains that can resist at least one of the only 100 or so antibiotics currently available.*
> *-In 1992, 13,300 hospital patients died from bacterial infections that resisted every drug doctors tried.*
> *-Bacteria can pass on their genes for resistance to 16,777,220 progeny every day [if a single bacteria divided once every hour for 24 hours].*
> *-Through conjugation, bacteria can give the DNA code for resistance to any bacteria it comes in contact with.*
>
> (Escalante *et al*., 1995)

From this data, the exponential increase of conjugation-spread antibiotic resistance is justifiably frightening. A recent estimate of the total number of bacteria on Earth is 5×10^{30} cells (Tenenbaum, 1998). Within this staggering number of bacteria, a mutation occurs roughly every 20 minutes (Tenenbaum, 1998). While only a very small fraction of the earth's bacteria are pathogenic to humans, the coupling of a high mutation rate with an immensely rapid reproduction rate is a good reason for why the spread of antibiotic resistance might be a problem.

Antibiotic resistance will *always* be a problem—there can be no end for several reasons. First, researchers are simply unable to develop new antibiotics at the same rate at which bacteria develop resistance mutations. Second, living things will always tend to evolve under the selective pressures of natural or artificial selection brought about by a change in the environment. In the case of bacteria, improper antibiotic use can cause selection for "the more resistant members of a population and…eliminat[e] the patient's indigenous flora, which might otherwise compete with the pathogen" (Yates, 1999). Third, a study has shown that there is a trend toward a higher incidence of resistance in immunosuppressed patients, persons who often *require* antibiotic treatment because of their infection-prone condition (Ewig *et al.*, 1999). Fourth, the rate of antibiotic resistance can rise if antibiotic consumption increases. For example, increased "macrolide consumption…led to a significant increase in the resistance rate of Streptococcus pyogenes to these antibiotics. A similar increase in macrolide resistance was observed in Finland" (Cizman, 1999). Finally, even if bacteria are blocked from conjugating, there is always a chance that a bacterium will develop a mutation that would circumvent the blockage. For example, an enzymatic GTD might be developed that blocked conjugation by destroying pili—the surface structures that join and fuse together when two bacteria conjugate, allowing genetic information to flow across. However, a random mutation might alter the pili binding site where the enzymatic GTD attaches, preventing the GTD from attaching. In this way, a bacterium *can* develop resistance to a GTD, which explains why GTD drugs will only be able to *slow* the spread of antibiotic resistance. However, even the smallest enhancement in antibiotic effectiveness offered by GTDs is well

worth investigating, since this enhancement may well translate into the saving of many lives otherwise lost to bacterial infection.

There are a vast number of conjugation steps that GTDs can inhibit, and currently, research has just touched upon a few of those steps. We now return to the conjugation-inhibition research group at the University of Arizona. This group has come up with five functions that pili-disabling GTDs could be designed to do. They include:

> *Competitive Inhibitor: A molecule that will bind to the pili reception sites of bacteria, thus preventing the conjugation of the bacterial cells*
> *Codon Mutation: A mutation in a codon resulting in a change of the primary sequence of the amino acids in the pili, hence preventing conjugation*
> *Enzyme: Create an enzyme that will destroy the pili or cleave the conjugation tube when it forms*
> *Genetic Engineering: Find and change the nucleic acid sequence of the DNA that makes the protein of the pili*
> *Dendrimer Molecule: Create a Dendrimer molecule that will bond to and encage pili*
>
> (Escalante *et al*., 1995)

Together, these functions should comprehensively stop conjugation from occurring, since if a GTD does not splice out the pili-coding sections of DNA to begin with, there are four more levels of inhibition. Inhibition by GTDs could occur during translation (when codon mutations are read), during conjugation (when enzymatic GTDs cleave conjugation tubes), or at any step in between, where molecules can encase pili to prevent them from becoming conjugation tubes, or bind to pili reception sites, preventing conjugation once more). Assuming that these GTDs do not interfere with normal eukaryotic chemistry, the redundancy of GTD activity demonstrated here should prove indispensable in greatly reducing the number of bacterial conjugations, the leading way in which antibiotic resistance is spread.

An important problem with designing gene transfer disabling drugs is that they will likely consist of molecules too large to enter through a bacterial cell wall. One way to solve that problem is if GTD drugs were made from nucleic acids—then, they could be injected into bacteria via the tiny syringes found on specialized *bacteriophage* tails. Bacteriophages are viruses that naturally use bacteria as hosts to grow and multiply. Because of this, they already have the intricate mechanical and chemical structures needed to inject nuclear material through bacterial cell walls. Bacteriophages (or simply, phages) come in many different shapes. These viruses identify and attach to specific bacterial surface receptors; engineered

phages could be made to attach to other structures that natural phages cannot. These engineered phages might include GTD-containing sections of DNA or RNA, so that once injected inside a cell, viral enzymes can splice these sections into the bacterium's DNA. Later, in bacterial translation, the GTD will be able to produce its desired gene transfer blocking effect. Along with adding GTD-containing sections, sections of nuclear material coding for viral replication would be removed. This would ensure that these engineered phages would not be able to redirect the cell's metabolism to build new virus particles. Preventing new phage production will be highly beneficial, since this would reduce the chance that engineered phages would mutate and infect other bacteria beside the ones they were designed to infect.

There are a variety of bacterial surface receptors that bacteriophages can attach to, some of which may be found on only particular species.

> *By diffusion phage particles will come into contact with surface receptors on the bacterial cell wall. In the case of bacteriophage T4 the bacterial cell surface receptors are LPS (lipopolysaccharides. . .) interacting with the proteins of the phage tail fibres. Other phage particles have different attachment sites such as F-pili (M13, fd) or flagella.*
> (Keil, 1995)

Because engineered phages might be able to binding to species-specific receptors, a GTD-containing section of nuclear material can be added to phage DNA or RNA, and will only have effects on the single species of bacteria infected by the bacteriophage. For the first time, the effects of a drug might be able to selectively target a single species. This is useful because gene transfer between non-infectious, beneficial bacteria would not be blocked, which will allow natural gene-sharing and evolution of these bacteria to continue. Minimizing the amount of disturbance to bacterial ecology is important when trying to counteract antibiotic resistance, as Spratt explains:

> *The eradication of the normal flora by powerful antibiotics has also led to the rise in prominence of intrinsically antibiotic-resistant species that previously rarely caused disease, but which can flourish when the microbial ecology is grossly disturbed.*
> (Spratt, 1996)

The need for GTD drug research is strong. According to a report by Hancock, despite the intensive research done by private industry,

> *…no novel chemical class of antibiotics has been discovered in the past 20 years… Indeed, all recently introduced antibiotic compounds are permutations (i.e. improved versions) of preexisting compounds. Thus, a situation whereby bacteria can mutate known resistance mechanisms to combat these improved*

> *relatives of earlier antibiotics has been created, and it is not unusual for significant resistance to be observed even before introduction of such antibiotics into the clinic.*
> (Hancock, 1997)

Developing new antibiotics would essentially prove futile if their intended targets have already developed resistance to them. Therefore, the first step in successfully treating bacterial infections should be to stop resistance from occurring in the first place. If GTD drugs can do this, then their development should certainly be worth investigating. GTD development is not out of reach—as demonstrated by the University of Arizona's conjugation-inhibition research group, the widespread use of GTDs may soon become reality.

The proper resources and technology *are available* for developing GTD drugs. First, the genomes of many types of bacteria have recently become fully sequenced, meaning that the genetic information which governs the biochemical steps of conjugation is readily accessible. Second, instruments exist that can resolve the molecular structure of individual proteins and other bacterial surface structures. This will make possible the identification of suitable receptors, some which are perhaps species-specific, to which engineered GTD-containing bacteriophages might attach. Third, software exists that will allow scientists to create drugs that will bind to specific cell surface structures. Pharmacologists at ViroPharma have recently used such technology to create *pleconaril*, an antiviral drug that has recently become the first to treat the common cold (ViroPharma, 2000). If a drug can be designed to attach to tiny viral surface structures, then certainly a drug can be designed to attach to one of the numerous types of receptors found on the surface of bacterial cells. If all of these current scientific and technological resources were properly used together, researchers should finally be able to create gene transfer disabling drugs.

Again, if fewer bacteria become resistant to antibiotics used in treatment, those antibiotics can be used for a longer time before antibiotic resistance renders those antibiotics ineffective. Using gene transfer disabling drugs will help to slow the pace of antibiotic resistance spread, and give researchers more time to develop new antibiotics. This means that many more lives would be saved, instead of being lost to resistant bacterial infections that largely could have been prevented.