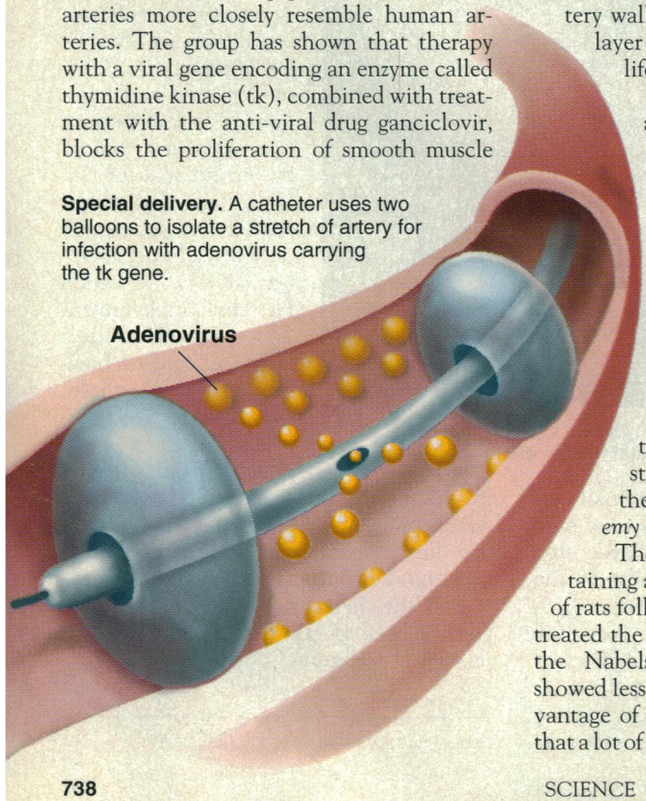
CARDIOVASCULAR RESEARCH

Gene Therapy for Clogged Arteries Passes Test in Pigs

Cardiologists have known for a long time that abnormal growth of the smooth muscle cells lining artery walls plays a key role in the blockage of arteries during coronary artery disease. It also contributes to reblockage of many arteries that have been opened by balloon angioplasty or replaced in bypass operations. This reblockage or "restenosis" is a "huge clinical and economic problem," says University of Chicago cardiologist and molecular biologist Jeffrey Leiden.

As befits a huge problem, many research groups are racing to solve it. Some of that work seeks to understand how restenosis happens (*Science*, 15 July, p. 320). But much of it is directed at treatment, and some of the newest work consists of attempts to use gene therapy to stop smooth muscle cell proliferation following balloon angioplasty. On page 781, a research team headed by Gary and Elizabeth Nabel of the University of Michigan Medical Center in Ann Arbor reports the most significant progress yet on the gene-therapy front.

Much of the research on restenosis has been done on rats, but those results may not be directly applicable to humans, since several remedies that work in rats have failed in human trials. The Nabel group, however, has chosen to work with pigs—an animal whose arteries more closely resemble human arteries. The group has shown that therapy with a viral gene encoding an enzyme called thymidine kinase (tk), combined with treatment with the anti-viral drug ganciclovir, blocks the proliferation of smooth muscle cells following balloon angioplasty in pigs. The Nabels "have fulfilled the fantasy that gene therapy can work in this condition," says Stanford University cardiologist Victor Dzau, "and they have used a different [animal] model than the rat, one which allows extrapolation to humans." Their approach may someday lead to a way of preventing restenosis that could be delivered at the same time as angioplasty; bypass grafts might be treated in the same way before being sutured into place.



Special delivery. A catheter uses two balloons to isolate a stretch of artery for infection with adenovirus carrying the tk gene.

SOURCE: GARY AND ELIZABETH NABEL ILLUSTRATION: K. SUTLIFF

For the current experiment, the Nabel team first performed balloon angioplasty on the groin arteries of pigs, an insult that triggers smooth muscle cell proliferation in an otherwise healthy artery. Then they used a modified form of a human respiratory virus called adenovirus to carry the tk gene into the damaged arterial cells.

The tk enzyme doesn't harm the proliferating cells directly, but it makes them susceptible to ganciclovir. The drug, which is also harmless by itself, is phosphorylated by the tk enzyme, causing it to insinuate itself into the DNA of dividing cells and halt cell division. Using this strategy, the Nabels' team found that, compared to control animals, the pigs treated with the tk gene and ganciclovir had a 50% to 90% reduction in artery wall thickening, specifically in the layer where smooth muscle cell proliferation occurs.

Encouraging as these results are, Chicago's Leiden notes that no animal model is a true replica of the human condition. "It is important to validate in a couple of models," he cautions. "That will make us a lot more confident in taking these things to humans." Such confirmation may already be in hand in the form of work by Toren Finkel, Stephen Epstein, and their colleagues at the National Heart, Lung and Blood Institute (NHLBI) that is in press at the *Proceedings of the National Academy of Sciences*.

The NHLBI team applied a tk-containing adenovirus to the carotid arteries of rats following balloon angioplasty, then treated the animals with ganciclovir. As in the Nabels' work, the treated animals showed less artery wall thickening. "The advantage of what [the Nabels] have done is that a lot of people would agree the pig model is more closely related to the human than the rat," says Finkel. But he adds that getting similar results in two systems "makes you think it is not just a weird artifact."

While that may be reassuring, there are several hurdles to be cleared before human trials can be considered. "The next major advance will require studies in the atherosclerotic setting," says Kenneth Chien, a cardiologist and molecular biologist at the University of California, San Diego. He notes that the pigs and rats tested so far had healthy arteries until balloon inflation caused smooth muscle cell proliferation. The technique might not work as well in arteries that are already atherosclerotic.

Researchers will also have to solve a technical problem for delivering the therapy before the technique can be used on coronary arteries. To keep the adenovirus from being swept away by the bloodstream before it could enter the smooth muscle cells, the Nabels isolated the injured area with a specialized catheter that has two balloons, one at each end. The tk-containing virus was injected between the balloons. But this blocked the blood flow in the pigs' leg arteries for 20 minutes. While the leg can handle such a blood-flow blockage, the heart can't, and so researchers will have to develop a catheter that shunts blood from one side of the closed-off artery section to the other.

Finally, there are safety considerations. Among other things, the safety of the thymidine kinase–containing adenoviruses will have to be established, although Elizabeth Nabel points out that the answer may come from clinical trials of gene therapy for some types of metastatic cancer, where such viruses are already being tested.

As an approach to restenosis, tk gene therapy is hardly alone. Several labs, including those of Leiden, Stanford's Dzau, and Robert Rosenberg at the Massachusetts Institute of Technology, have blocked smooth muscle cell proliferation in rats by targeting proteins that regulate cell division, using either gene therapy or "anti-sense" nucleic acids that block expression of targeted genes.

And there may be other ways of preventing restenosis in addition to blocking smooth muscle cell proliferation. One such possibility: giving the gene for the enzyme nitric oxide synthase, whose product, nitric oxide, acts to relax blood vessels and prevent clot formation. The best chance of clinical success, says Finkel, may be with a cocktail of genes that affect different aspects of restenosis. Indeed, agrees Dzau, "the exciting thing about all this is that after all these years, we are beginning to find out that it is possible to inhibit at least some of the processes that lead to restenosis." And, he says, that brings hope that "eventually we can put it all together to make it work in humans."

–Marcia Barinaga