Researchers gain new clues about how to prevent aortic aneurysm in patients with Marfan syndrome

April 14 2011

Five years ago, patients with Marfan syndrome received new hope when laboratory studies suggested that losartan, an FDA-approved drug used to treat high blood pressure, might prevent the potentially deadly enlargement of the aorta that the syndrome can cause. Now, researchers have a clearer picture of the cellular signals that contribute to progression of aortic aneurysm in Marfan syndrome and how losartan alters those signals. The new information is expected to help guide treatment decisions, as well as efforts to develop therapies that might offer benefits that losartan does not.

The new work by Harry C. Dietz, a Howard Hughes Medical Institute investigator at the Johns Hopkins University School of Medicine, builds on his team's earlier discovery that blocking signaling from a molecule called TGF-beta halts the progression of aortic aneurysm in a mouse model of Marfan syndrome. Losartan dampens TGF-beta signaling by blocking one of its partners, the angiotensin II receptor. Dietz's new work, described in two papers published April 14, 2011, in the journal *Science,* clarifies which molecules in the cell work with TGF-beta to drive aneurysm progression.

The findings have implications both for how losartan works relative to other existing drugs, and for how researchers might focus future drug development efforts. "I really think we're going to be able to make more informed choices regarding which medication to use now [to protect

against aortic aneurysm], and which medications to test in the future," Dietz says.

Based on Dietz's earlier work, the National Heart, Lung, and Blood Institute launched a clinical trial in 2007 to test whether losartan could slow progression of aneurysm in people with Marfan syndrome. Dietz reports that the phase III trial has completed its enrollment of 604 patients. Those patients will be followed for three years, and like many in the Marfan community, Dietz is optimistic. "Losartan is truly amazing at suppressing aneurysm progression in the mouse model," he says. "But even though losartan looks great in animal models, we still have to prove that it's going to be as good -- or good at all -- in people with Marfan syndrome."

There's more reason to be cautious, he says. Even if it works for most patients, losartan is not appropriate for everyone. Its blood pressure-lowering effects, for example, are not desirable for some patients, and the drug cannot be taken during pregnancy. Furthermore, Dietz adds, while the preclinical evidence suggests losartan could stymie Marfan's effects on the aorta, patients with the syndrome face other, less severe, symptoms that might be treated more effectively with other therapies.

For these reasons, while the losartan trial is underway, Dietz's lab is continuing to aggressively seek more detailed information about the cellular underpinnings of Marfan's symptoms. The surprising finding that TGF-beta signaling can drive aneurysm progression in mice offered the initial rationale for testing losartan in patients with Marfan syndrome. Now Dietz hopes an even more intricate understanding of that signaling could lead to better or alternate therapies.

TGF-beta is involved in a variety of processes in healthy cells. It helps control cells' growth, development, and even death, and it does so by sending signals via several different pathways. So when TGF-beta

behaves inappropriately, there are lots of ways in which things can go wrong.

TGF-beta exerts many of its effects through a pathway that relies on a family of proteins called Smads. Smad signaling has come to be thought of as the "canonical" TGF-beta pathway, but it does not work alone. Dietz thought it was important to figure out which TGF-beta pathways were contributing to Marfan's effects on the aorta. "Every manipulation that caused aneurysms to get better were associated with reduced activity of Smads," Dietz acknowledges, but he wanted more than circumstantial evidence.

His team focused first on non-canonical TGF-beta signaling, looking to see if molecules in these pathways behaved unusually in the aortas of Marfan mice. Their most striking observation was the excessive signaling by a molecule known as ERK. They confirmed that ERK's activity depended on TGF-beta and the angiotensin II receptor, and that convinced them to try shutting off ERK in the Marfan mice.

Using an ERK inhibitor that is currently being developed as a possible cancer therapy, the scientists blocked the ERK signal. The treatment, they found, was "every bit as effective as losartan in suppressing abnormal aortic growth," Dietz says.

The team still wondered to what extent Smad-dependent signaling contributed to aortic growth, so they next planned to genetically inhibit Smad signaling in the Marfan mice. "If Smads were important, we expected the animals to get better," Dietz says. But things didn't go as expected. The mice got worse. "Indeed, they got much worse," Dietz says, explaining that the animals' aneurysms grew more quickly, caused more damage, and accelerated death.

It turned out that instead of inhibiting Smad, their genetic manipulations

had tweaked a third TGF-beta pathway. They had increased signaling along a pathway that relies on a molecule called JNK. So the team followed that accidental lead, and tried shutting JNK back off. When they did so, the animals got better: they overcame not just the excessive JNK signaling caused by the experimental misadventure, but also the Marfan mutation, and lived with healthy aortas. Even more dramatically, the team saw the same improvement when they blocked JNK signaling in Marfan mice in which they had not genetically altered JNK signaling.

"That suggested to us that both non-canonical cascades, ERK and JNK, somehow work together to drive aneurysm progression," Dietz says. And based on the improvements they saw when they blocked either of these pathways in the mice, it looked like "anything you did to knock down the total level of non-canonical activation would lead to therapeutic gain."

"So now we have two new therapeutic targets for Marfan syndrome," Dietz says. He points out that because JNK and ERK are close to the "disease-specific event," blocking them with drugs might trigger fewer unwanted side effects than therapies that interfere with signals higher up in the pathway.

Dietz says the new information will be useful for monitoring the effectiveness of drugs in ongoing and future clinical trials; changes in activity of the JNK and ERK pathways can be considered indicators of how a drug impacts the most relevant aspects of TGF-beta signaling. Additionally, he says, the study highlights a new set of genes and pathways that might contribute to the many aneurysm conditions that are more common, but less understood than Marfan syndrome.

In a separate set of experiments, the team scrutinized the angiotensin II receptor, the cell surface molecule targeted by losartan. The hormone angiotensin II activates cell signaling by binding to either of two forms of this receptor. Activation of the type 1 receptor (AT1) promotes TGF-

beta signaling, and many researchers agree that this has a detrimental effect on the aorta. AT1 is the form of the receptor blocked by losartan.

The role of the type 2 receptor (AT2), however, had been less clear. Because AT2 can promote programmed cell death, some scientists suspect that like AT1, it has detrimental effects on the aorta. But others, including Dietz, thought the evidence suggested that AT2 actually protects the heart.

Dietz says there is evidence that signaling from AT2 might help overcome the detrimental effects of AT1 signaling. If that's the case, blocking only the AT1 receptor (as with losartan) might spur the protective effects by forcing more of the angiotensin II hormone to bind to the AT2 receptor. But others have argued that the class of drugs known as angiotensin-converting enzyme (ACE) inhibitors, which limit production of angiotensin II and thereby reduce signaling through both pathways, might be better. The result, Dietz says, was "a raging clinical controversy."

To settle the debate, Dietz's team engineered a Marfan mouse that lacked the AT2 receptor. The results were clear. "We found that Marfan mice lacking the AT2 receptor do worse in every way," Dietz says.

Compared to the mice with only the fibrillin-1 mutation, those lacking AT2 had larger aneurysms and more aortic destruction at every age, and died earlier due to aortic rupture. They even had aneurysms in segments of the aorta that are typically spared in Marfan mice, Dietz says. Losartan did little to improve symptoms in mice lacking the AT2 receptor, which Dietz says supports the idea that losartan shunts signaling through a protective receptor. AT2, he says, is "actually part of the solution." The researchers went on to show that AT2 achieves protection by blocking activation of ERK.

The team then compared the effects of treating Marfan mice with losartan and the ACE inhibitor enalapril. Again, they found dramatic differences. Aneurysms in the mice that were treated with losartan got smaller over time, but in the animals treated with enalapril -- which reduces signals through both AT1 and AT2 -- aneurysm growth slowed, but remained accelerated compared to mice without Marfan syndrome. While losartan completely shut ERK down, enalapril had no effect on ERK.

Dietz says the clinical implications are clear. "I really think that our results provide firm rationale for the use of angiotensin-receptor blockers as opposed to ACE inhibitors," he says. "Right now patients are really caught in the middle with people arguing using purely theoretical considerations. Now we have data to support the greater potential of one class of medications versus another class."

Dietz adds that the findings should also be of interest to physicians treating other cardiovascular conditions. Like losartan, enalapril is currently used primarily to treat high blood pressure, and Dietz says ACE inhibitors and drugs that block the angiotensin receptor directly have often been considered more or less interchangeable. "But now we've demonstrated that these drugs have discrete effects, and provided a precise mechanistic explanation for why this is so."

Provided by Howard Hughes Medical Institute