Study establishes major new treatment target in diseased arteries

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Removing a single protein prevents early damage in blood vessels from triggering a later-stage, frequently lethal complication of atherosclerosis, according to research published online today in the journal *Nature Medicine*. By eliminating the gene for a signaling protein called cyclophilin A (CypA) from a strain of mice, researchers were able to provide complete protection against abdominal aortic aneurysm (AAA).

The aorta is the main artery carrying blood from the heart, and AAA is a progressive outward dilation of the aorta under the stress of blood pressure due to a breakdown in the vessel's structural integrity. AAA leads to 15,000 deaths a year, mostly in aging men, when aneurysms rupture to spill blood into the abdomen, a fatal event in 90 percent of cases. Adding to the study's importance, AAA shares vital biochemical pathways with atherosclerosis, the leading cause of heart attack and stroke. Thus, drugs that target CypA could potentially address both AAA and atherosclerosis.

When study mice were engineered to remove their CypA gene, none from that group developed AAA in the face of the hypertension and high cholesterol known to accelerate it. In contrast, 78 percent of mice with "normal" amounts of CypA developed AAA under the same conditions, 35 percent with a fatal rupture. The team also found high CypA levels in the rupture-prone vessels of humans with AAA, and that major drugs like statins reduce CypA levels, which may partly explain their benefit.

"It is extremely unusual for the removal of one protein to provide

absolute protection, but it makes perfect sense because cyclophilin A promotes three of the most destructive forces in blood vessels - oxidative stress, inflammation and matrix degradation," said Bradford C. Berk, M.D., Ph.D., professor of Medicine within the Aab Cardiovascular Research Institute at the University of Rochester Medical Center, and senior author of the study. "We are working to design anti-CypA drugs that will diminish the disease processes underlying AAA, atherosclerosis and hypertension."

Stress Relief

While a complete understanding of the initial triggers of blood vessel damage remains elusive, damaged vessels produce more reactive oxygen species (ROS), molecules that oxidize many molecules they encounter, changing their function. Human cells have evolved to harness oxidation to control life processes like wound healing, and to send signals within cells and between cells. The theory gaining strength since the early 1990s is that disease-related overproduction of ROS (oxidative stress) damages organs by destroying cell components, triggering cells to self-destruct and promoting inflammatory cell signaling.

Among the hormones best known to stimulate oxidative stress in blood vessels is angiotensin II. In AAA, past studies have confirmed that angiotensin II, in part by driving up ROS production, excessively turns on enzymes called matrix metalloproteinases (MMPs). As part of normal healing, MMPs chew through strong flexible proteins like elastin that give tissues shape (extracellular matrix) to make room for new growth. That process goes too far in an aneurysm, as ROS drive MMPs to degrade the matrix structure of the vessel wall. In atherosclerosis, overactive MMPs digest the structural barriers in vessel walls that contain smooth muscle cells, which frees them to contribute to vessel-clogging plaques. When blood vessels eventually become completely blocked, heart attacks and strokes occur.

Also as part of both AAA and atherosclerosis, angiotensin II causes immune cells to home in on the blood vessel wall in the process called inflammation. While designed to fight infection, immune cells often mistakenly contribute to disease, in some cases by secreting chemicals called cytokines that kill cells outright, and that also signal for more immune cells to home in on the site of damage or infection.

The current study sought to answer the question: can angiotensin II achieve these disease-causing effects if CypA is not there to pass on its message? To clarify the role of CypA, Berk's team engineered mice to no longer produce apolipoprotein E, which increased their cholesterol levels and made them prone to atherosclerosis. From this original line, the team further engineered one group with no CypA, another with extra CypA and compared both to "normal" mice as all were treated for a month with angiotensin II (known to raise blood pressure and accelerate AAA).

Mice lacking CypA saw greater than 75 percent decreases in ROS production, MMP activation and inflammatory cell influx compared to normal mice, with the opposite being true for mice with extra CypA. Angiotensin II treatment also dramatically increased expression of cytokines (e.g. monocyte chemoattractant protein 1(MCP-1)), unless CypA was missing.

"Our lab has been studying CypA since the early 1990s," Berk said. "We had to determine that vessel walls were secreting something in response to ROS, then prove it was CypA, then prove CypA was required for oxidative stress and inflammation to take their toll in live animals. Our results should put an end to debates within the field and pharmaceutical companies about whether we have found a vital new role for this well known molecule. Incredibly, CypA is required both inside and outside of cells to promote angiotensin II-mediated pathogenic effects in vessel walls."

Berk and colleagues propose that ROS generated via angiotensin II trigger CypA secretion from smooth muscle cells in vessel walls. Once outside the cell, CypA docks into CypA receptor proteins on the same cells to increase ROS production in a vicious cycle. When a signaling molecule docks into its receptor, like a key turning a lock, it changes the receptor's shape such that signals get passed on. Most drugs work by interfering with receptors, and Berk's team is searching for the specific CypA receptors that, if interfered with, would shut down ROS production, CypA secretion, MMP activation and inflammatory cell recruitment in AAA. Also in the next phase, Berk expects to complete a study shortly that will confirm CypA deficiency significantly slows the progression of atherosclerosis.

Along with first author Kimio Satoh, M.D., Ph.D., and Berk, the paper was co-authored by Tetsuya Matoba, M.D., Ph.D.; Michael O'Dell, B.S.; Patrizia Nigro, Ph.D.; Zhaoqiang Cui, Ph.D.; Xi Shi, Ph.D.; Amy Mohan, B.S.; Chen Yan, Ph.D.; Jun-ichi Abe, M.D., Ph.D. and Karl Illig, M.D., all within the Aab CVRI and the University of Rochester School of Medicine and Dentistry. The work was supported by the National Heart, Lung and Blood Institute, part of the National Institutes of Health, and by the Japan Heart Foundation.

"Currently available and experimental therapies, including ACE inhibitors and antagonists of the angiotensin receptors, MCP-1 and MMPs have significant limits in terms of efficacy in AAA, and thus, CypA inhibitors have the potential to meet significant unmet need," Berk said. "Additionally, inhibition of CypA looks to have tremendous benefit in several diseases that involve blood vessels in the brain and heart. Furthermore, while drugs that inhibit CypA may overlap somewhat with other drugs targeting the same angiotensin II pathway, they also look to have additive effects that create potential for combination therapies."

Source: University of Rochester Medical Center (news : web)