A new model takes oxidative stress to heart

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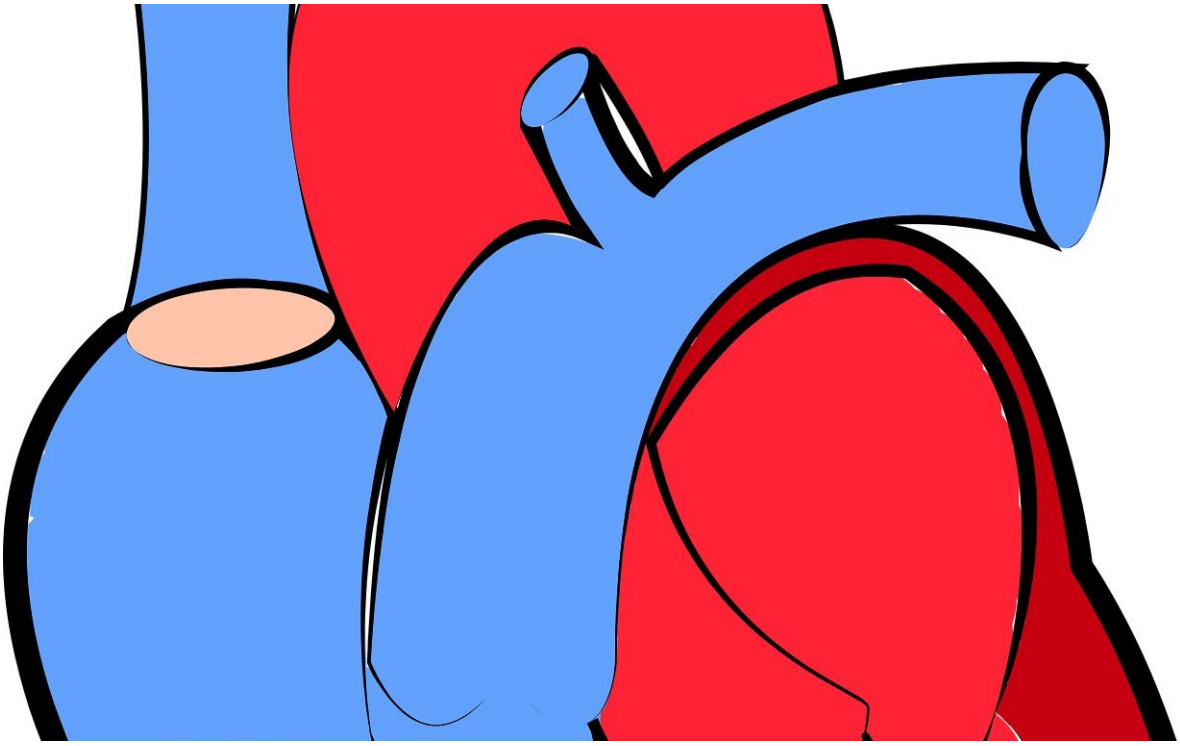

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Oxidative stress—the molecular wear and tear that reactive oxygen species can exert on molecules and cells—has been linked to a range of human diseases, including heart failure and Alzheimer's disease. But the results of many clinical trials that tested the effects of combatting oxidative stress with simple remedies—such as vitamin supplements or blueberries—have painted a more complicated picture. For many years,

researchers have been trying to tease out the nuances of the connections between oxidative stress and disease. But the diseases associated with oxidative stress typically involve multiple pathways in the body, and it has been challenging to define the specific roles of oxidative stress in disease progression at a molecular level. Investigators from Brigham and Women's Hospital have developed a robust new method for examining oxidative stress in the hearts of rodents in vivo to better understand the development and treatment of heart failure. Results of their novel methodology, applying a cutting-edge approach known as "chemogenetics," are published this week in *Nature Communications*.

"This is a new heart failure model that allows us to specifically study a critical player in heart disease: oxidative stress. This is the first time that we've been able to prove definitively that oxidative stress is a cause of heart failure," said Thomas Michel, MD, Ph.D., senior physician in the Brigham and Women's Hospital (BWH) Division of Cardiovascular Medicine and professor of medicine at Harvard Medical School.

Chemogenetics is an approach that allows researchers to activate or inactivate a recombinant protein in cells or tissues simply by providing or withdrawing the specific molecules that bind to the protein. The BWH researchers developed an in vivo method using this approach that allowed them to generate and measure a reactive oxygen species —hydrogen peroxide—specifically in the heart, and then monitor the onset of cardiac dysfunction. Michel's team is using this approach to create a more tractable preclinical model of heart failure to help increase the speed and scope of drug development and testing.

"A lot of the models of heart failure that have been used for drug development have moved forward one animal at a time," said Michel. "But with chemogenetics, a path is opening up for high-throughput screening of new heart failure drugs."

To examine oxidative stress on the heart, the research team took advantage of a D-amino acid oxidase (DAAO) - an enzyme that was cloned from yeast. DAAO is known to generate hydrogen peroxide (H2O2) only in the presence of D-amino acids, leading to oxidative stress. But mammalian cells use L-amino acids, not D-amino acids: a subtle but important difference that allows the introduced yeast enzyme to remain quiescent until it's provided with its D-amino acid substrate. The team used a virus to deliver DAAO to the hearts of rats, and then the animals were provided with drinking water containing a D-amino acid in order to activate the DAAO. After 4-5 weeks, the team examined hearts using echocardiography to measure cardiac function and heart size. Additionally, the researchers measured markers of inflammatory and adaptive stress.

Compared to animals that received a control virus, the rats expressing DAAO showed signs of advanced heart failure, including increased heart size and decreased contractile function, which were specifically caused by oxidative stress in the heart.

"We anticipate that chemogenetic approaches will enable future studies not only in the heart, but also in the many other organ systems where the relationship between redox events and disease remains unclear," said co-lead author Ben Steinhorn, a Harvard MD-Ph.D. student who pursued his Ph.D. in Michel's lab.

"Heart failure can now be examined in a rapid, reliable, and reversible manner," said co-lead author Andrea Sorrentino, a BWH postdoctoral research fellow. "Instead of doing surgery one animal at a time, we can deliver the virus to many animals at once, and then activate the DAAO enzyme just by providing its substrate in their drinking water to trigger specifically oxidative stress in the heart."

With funding from the Brigham and Women's Hospital Health and

Technology Innovation Award, Michel's lab is also developing a transgenic mouse—an animal model whose genome has been altered to encode DAAO, allowing researchers to skip the viral vector and more easily study oxidative stress.

More information: Benjamin Steinhorn et al, Chemogenetic generation of hydrogen peroxide in the heart induces severe cardiac dysfunction, *Nature Communications* (2018). DOI: 10.1038/s41467-018-06533-2

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