Mini heart attacks lessen damage from major ones

December 2 2008

Researchers have discovered one potential mechanism by which briefly cutting off, then restoring, blood flow to arteries prior to a heart attack lessens the damage caused, according to a study published today in the journal *Cardiovascular Research*. The new mechanism points to how future drugs could provide protection ahead of heart attacks and strokes for those at highest risk. In the nearer term, the work may help to prevent damage caused as U.S. heart surgeons temporarily cut off blood flow 450,000 times each year to perform coronary artery bypass graft surgeries. Lastly, the discoveries hold clues to the value of the Mediterranean diet beyond red wine.

In severely diseased coronary arteries, fatty deposits in blood vessel walls become more likely to rupture, which releases proteins into the blood that cause blood clots and cut off blood flow. When a vessel becomes completely blocked (ischemia) the downstream tissue begins to die for lack of oxygen and nutrients. Worse yet, when blood flow is restored (reperfusion), the returning blood throws off cellular chemistry, creating as a side-product a burst of highly reactive "free radicals" that tear apart cell components and cause cells to self-destruct. Later in the process, the immune system attacks the cardiac tissue damaged by ischemia and reperfusion, causing inflammation which can lead to heart failure.

In 1986 then medical student Chuck Murry at Duke University first described a technique called ischemic preconditioning (IPC), which quickly cuts off then restores blood flow to the heart. He found that IPC somehow protected heart tissue against the damage caused by

subsequent, prolonged blood vessel blockages. An emerging theory holds that this natural early warning system of IPC has evolved to protect against heart attack. Labs worldwide are seeking to re-create or strengthen this natural protection against ischemia/reperfusion (IR) injury. In the current study, researchers for the first time determined that IPC caused more of a key molecule, nitro-linoleic acid (LNO2), to be made in ischemic cells.

"LNO2 appears to be important in the mechanism by which IPC triggers the body's natural defense mechanisms against heart attack before the major attack comes," said Paul S. Brookes, Ph.D., associate professor of Anesthesiology and of Pharmacology and Physiology at the University of Rochester Medical Center. "Obviously, this natural response, when it follows a major heart attack, is often too little too late. Our hope is that boosting the effect in patients at high risk, perhaps by administering LNO2 beforehand, will reduce heart attack damage in the future. Even sooner, we may be able dramatically reduce reperfusion injury suffered in surgical settings."

Going into the study, the mechanisms underlying IPC protection were controversial, but a consensus had emerged recently that mitochondria were involved. The cell's powerhouse, mitochondria use oxygen to convert nutrients into cellular energy supply.

As they do so, they create a gradient of protons across their membranes. When the gradient becomes too large, it triggers the mitochondria to use oxygen to generate free radicals. The problem gets much worse when blood returns to a vessel after a blockage, bringing with it a surge of oxygen and nutrients.
It has long been thought that a group of proteins in the mitochondrial membrane act as a "safety valve" by dissipating too large proton gradients when necessary, which slows free radical generation. The current study identified a novel mechanism involving LNO2, by which

IPC turns on this safety valve.

Given their results, the authors propose the following protective mechanism: temporary ischemia causes the generation of nitrated lipids inside the mitochondria via currently unknown mechanisms involving metabolites of the gas nitric oxide (NO). These lipids, including LNO2, then become attached to two proteins – adenine nucleotide translocase and uncoupling protein 2 – changing their shape such that they allow a proton leak across the mitochondrial membrane. The leak lowers the proton gradient just enough to lessen free radical production.

While the current study only looked at the immediate effects of LNO2 treatment, the literature suggests that LNO2 also limits the misplaced immune response seen after reperfusion, suggesting a dual treatment effect. Past studies found that LNO2 inhibits Nfkappa B, a protein known to switch on genes that drive inflammation. LNO2 also activates peroxisome proliferator activated receptor gamma and heme oxygenase 1, both of which block inflammation.

The major finding of the study is that LNO2 is formed naturally in mitochondria during IPC in an isolated rat heart, and that adding extra LNO2 protects heart muscle cells from IR injury. The team measured the ability of isolated rat heart cells to survive ischemia using a dye that the live cells keep out, but that dead cells take in. That enabled researchers to count how many cells survived with and without LNO2 added.

In normal cells following ischemia 70 percent died, but for those receiving extra LNO2 (0.5 micromolar), only 30 percent died. The amount of the LNO2 added was not much more than naturally occurs, suggesting its effect is "extremely potent," researchers said. The LNO2-related proton leak also occurs at the protein level within seconds, a vital quality of any future therapy, considering that IR injury greatly

increases with each second it is allowed to proceed.

Brookes is part of the Mitochondrial Research & Innovation Group (MRIG) at the Medical Center, which last year reported in the Journal of Molecular and Cellular Cardiology on the design and testing of a series of patented nitric oxide donors that break down and release NO only within the mitochondria, and protect the heart from ischemia. The team believes these NO donors may work in part by increasing LNO2 supply. In the acute setting, such drugs may offer an advantage over standard nitric oxide donors like nitroglycerin, which increase blood flow in diseased arteries by causing them to dilate throughout the body. Standard NO donors also depress cardiac function by decreasing the pressure of blood returning from the body back into the heart. Early tests in a mouse model have confirmed that the new MRIG NO donor drugs are cardioprotective in-vivo, and do not cause system-wide vessel dilation side effects.

Bruce Freeman, Ph.D., chair of the Department of Pharmacology & Chemical Biology at the University of Pittsburgh School of Medicine, also led the study. Other contributors included postdoctoral fellow Sergiy Nadtochiy, Ph.D. in Rochester, and Paul Baker, Ph.D. research assistant professor in Pittsburgh.

"Our interest in this area stems from the fact that many different stimuli appear to funnel down into the mitochondria where they may trigger LNO2 production, any of which may suggest a new way to prevent damage," Freeman said. "Along with IPC, olive oil has been shown to produce LNO2 in the stomach, offering an explanation for the value of the Mediterranean diet."

Source: University of Rochester