Researchers unravel key mechanism of cellular damage in aging and disease

July 24 2008

Researchers have taken a first snapshot of how a class of highly reactive molecules inflicts cellular damage as part of aging, heart disease, stroke, cancer, diabetes, kidney disease and Alzheimer's disease to name a few. According to a study published today in the journal *Cell*, researchers have discovered a tool that can monitor related damage and determine the degree to which antioxidant drugs effectively combat disease.

Reactive oxygen species (ROS), which include free radicals, are highly reactive molecules that force change upon many molecules they encounter. The body uses ROS to signal for wound healing and to destroy invaders. Excess amounts, however, damage sensitive cell components, including proteins and DNA, in a process called oxidative stress. ROS are kept in check by the body's natural antioxidants, but when uncontrolled can lead to disease.

These highly reactive molecules are created as a side effect when structures within all human cells, the mitochondria, use oxygen to convert food into energy for life. Researchers once thought that altered mitochondrial function was important only in rare genetic diseases, but recent studies have revealed that oxidative stress plays a role in conditions that afflict many millions of patients. As a result, mitochondrial medicine is gaining momentum as groups like the Mitochondria Interest Group at the National Institutes of Health, professional societies and drug companies push basic science toward drug discovery.

"Our study provides a better glimpse of why a cell under assault by disease makes 10 times as many reactive oxygen species as the same cell when healthy," said Shey-Shing Sheu, Ph.D., professor of Pharmacology and Physiology at the University of Rochester Medical Center, and a study author. "We have discovered a chemical tool for investigating how diseases cause damage, mitochondrion by mitochondrion, which should represent a tremendous advance as both a disease biomarker and for drug discovery."

Meltdown at the Powerhouse

The primary role of mitochondria is to convert food into chemical energy in the form of adenosine triphosphate (ATP), the molecule used by all human cells to store energy. To drive ATP production, electrons are passed along a series of enzymes, but a few "leak" during the process. Leaked electrons react with available oxygen to form the reactive oxygen species, termed superoxide. The emerging theory is that excess superoxide production causes the mitochondria to swell and rupture, resulting in a "cellular energy crisis" that ultimately leads to cell death in the many diseases of oxidative injury.

In the Cell study, researchers used a newly patented, protein-based probe to discover, and make visible, for the first time, fleeting bursts of superoxide production in mitochondria termed "superoxide flashes." The superoxide bursts oxidize cysteine residues in the probe, causing it to emit fluorescent light, which can then be detected and analyzed for patterns. Experiments not only identified superoxide flashes for the first time, but also confirmed that they exhibit a similar size and duration, regardless of the cell type they occur in. This uniform pattern of low-level superoxide production in the mitochondria of healthy cells is normal, perhaps keeping the ROS signaling system ready to fire, researchers said. The one quality of superoxide flashes found to vary was frequency, which dramatically increased during disease.

The research team looked at one kind of oxidative stress in particular: that caused when the oxygen supply to the heart is initially cut off (e.g. during a heart attack or stroke) and then re-established. When heart muscle cells were exposed to a non-oxygen solution for six hours, superoxide flash frequency decreased four-fold, to one event per 100 seconds. Upon re-introduction of oxygen, superoxide production increased eight-fold, confirming past work that re-oxygenation after a heart attack comes with a burst of destructive and uncontrolled superoxide production and oxidative stress.

Existing methods for measuring superoxide production involve chemical indicators or older protein probes, but these previous methods are not specific for superoxide, are damaged by light and provide a limited signal above background noise. The new probe is more sensitive, specific to superoxide, provides a stronger signal than other probes, and is the first to permit reversible measurements of superoxide levels on a millisecond timescale. The research team also created a genetically engineered mouse that expresses the probe within the mitochondria of all of its cells. These "superoxide mice" will enable researchers in the future to quantify the impact of uncontrolled mitochondrial superoxide production across many diseases.

Efforts to develop antioxidant drugs (e.g. vitamin E) to treat diseases of increased oxidative stress have met with limited success to date because they tried to eliminate ROS, rather than maintain the right amount, Sheu said. He established the Mitochondrial Research & Innovation Group (MRIG) at the Medical Center in 2002 with the goal of designing therapies to deliver precise amounts of antioxidants to the mitochondria of diseased cells only. MRIG teams are, for example, screening through compounds to confirm that oxidative stress can be reversed by mitochondria-specific drugs. The new superoxide flash probe will provide a powerful tool for determining the effectiveness of new classes of antioxidant drugs in reducing superoxide production at the right place

and time.

The "birthday" for superoxide flashes came in June of 2003 in the lab of Robert Dirksen, Ph.D., associate professor of Pharmacology and Physiology at the Medical Center, when Linda Groom observed spontaneous bursts of fluorescent light using the newly developed protein-based superoxide indicator. The current paper's lead author was Wang Wang, Ph.D. formerly a postdoctoral fellow at the National Institute on Aging at the National Institutes of Health. Aiwu Cheng, Jinhu Yin, Weidong Wang, Edward Lakatta and Mark Mattson also contributed from the NIH, as did Joseph Kao from the University of Maryland. Also contributing from Peking University in Beijing were Huaqiang Fang, Wanrui Zhang, Jie Liu, Xianhua Wang, Kaitao Li, Peidong Han, Ming Zheng, and Heping Cheng, the corresponding author.

"One co-author on the current paper, Dr. Cheng of Peking University, 15 years ago published a seminal study regarding local calcium release events, or calcium sparks," Dirksen said. "This study has been cited more than 900 times and has provided a major contribution to our understanding how the heart beats and why it fails. We believe that our serendipitous discovery of local mitochondrial superoxide flash events could be even more important because superoxide flash frequency may provide an accurate, real-time picture of how uncontrolled oxidative stress contributes to the progression of several, debilitating cardiovascular and neurological diseases."

Source: University of Rochester