

Autophagy and Antioxidants



Dr. David Rubinsztein

A team of researchers at the University of Cambridge led by Dr. David Rubinsztein has explored the relationship between autophagy and the production of reactive oxidative species (ROS). They found that it is possible to induce autophagy without increasing toxic ROS and that some antioxidants reduce autophagy.

Autophagy, which literally translates as 'self eating,' is a very old cellular house cleaning process (it's found in organisms from yeast to mammals). Damaged parts of the cell, pathogens, and large proteins are surrounded by autophagosomes. The autophagosomes deliver their cargo to the lysosomes by fusing with them. The lysosomes then

consume the material. Researchers are interested in upregulating autophagy as a potential treatment for Huntington's Disease.

Autophagy is an alternate method of protein degradation. The normal huntingtin protein is degraded in the cytosol of the cell by the Ubiquitin Proteasome System (UPS) in which proteins that are not needed or that have misfolded are tagged for degradation by a small protein called ubiquitin. The unwanted protein is then moved into the proteasome, a barrel like protein complex, which breaks it down into amino acids that can then be recycled. However, in Huntington's Disease, the UPS is unable to handle the HD protein properly.

There are a number of issues still to be resolved. One issue was raised by researchers who found that that cargo recognition was impaired in Huntington's Disease and cellular garbage wasn't getting to the lysosomes. Another issue, which is addressed by Rubinsztein and colleagues, is whether autophagy can be enhanced without increasing reactive oxidative species.

Energy for cellular processes is produced in the mitochondria. During the process, ROS is produced. ROS are molecules of oxygen with only one electron, instead of two electrons that are bonded together which is the normal form of oxygen. This means they can go scavenge for other molecules to bond with. Normally this isn't a problem because nearby antioxidants will bond with the ROS molecules and render them harmless. When levels of ROS rise however, they cause oxidative stress, destroy cellular compounds, damage proteins, lipids, and DNA, and lead to cell death.

One pathway that induces autophagy is also known to increase ROS. Starvation induces autophagy but it also increases ROS. Rubinsztein and colleagues wanted to know whether all pathways to inducing autophagy would do the same. They looked at two types of non-HD cells (HeLa and COS-7 renal cells), adding rapamycin, an antibiotic,

and trehalose, a sugar, both of which have been shown to induce autophagy. Each increased autophagy but neither increased ROS.

Next they looked at the affect of various antioxidants on autophagy in the COS-7 renal cells. Autophagy was measured by levels of LC3-II, a marker for autophagosome formation and autophagosome-lysosome fusion. They looked at N-acetyl-cysteine (NAC), cystamine (in the pipeline as a potential HD treatment), and glutathione. All three impaired the induction of autophagy by trehalose in a dose dependent manner. They also looked at the effect of NAC and cystamine on rapamycin induced autophagy and on basal (normal, not induced levels of) autophagy and got similar results.

NAC, cystamine, and glutathione are all thiol antioxidants, organic compounds that contain a sulfur-hydrogen bond. The researchers next examined whether a non-thiol antioxidant also inhibited autophagy. They found that Vitamin E also impaired trehalose induced autophagy. They also upregulated the gene for SOD11 which scavenges superoxide. Upregulating SOD11 reduced basal autophagy.

The researchers examined the effects of antioxidants and autophagy in drosophila models of HD. A low dose of NAC alone did not exacerbate the disease but a high dose did. However, a low dose coupled with rapamycin reduced rapamycin's ability to rescue cells. This was also true for cystamine. Over-expressing SOD11 exacerbated the disease.

The researchers also found that NAC and vitamin E significantly increased the number of aggregates of mutant huntingtin in a zebrafish model of HD. Rapamcyine and clonidine, both autophagy inducers, decreased the aggregates but this affect was partially blocked by co-treatment with NAC.

NAC was also found to inhibit starvation induced-autophagy in the livers of mice.

Autophagy inducers are in the pipeline as are antioxidants and cystamine. This study shows how important to understand the mechanisms by which potential treatments work and how they interact with other potential treatments at various doses and at various points in the disease process. Because Huntington's Disease is a multi-hit disease, combination treatment will be necessary, at least until a treatment which interferes with the expression of the HD gene is available.

Reference:

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- *Marsha L. Miller, September 28, 2010*