Published in final edited form as:

Neurogastroenterol Motil. 2011 February ; 23(2): 111–114. doi:10.1111/j.1365-2982.2010.01659.x.

Oxidative Stress: Key player in Gastrointestinal Complications of Diabetes

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Keywords

Oxidative stress; diabetes; lipoic acid; HO1

Oxidative stress results from an imbalance between production of reactive oxygen species (ROS) and antioxidant defenses¹ such as catalase, superoxide dismutase and heme oxygenase. While ROS are important second messengers at low concentrations and are involved in regulating apoptosis² and activation of transcription factors such as NF- κ B³, they can cause significant cellular damage when present in excess. Oxidative stress has been implicated in several disorders of the cardiovascular system,⁴ in neurodegenerative disorders and in aging.⁵ More recently oxidative stress has been recognized as an important factor contributing to gastrointestinal dysmotility such as post-operative ileus,⁶ diabetic gastroparesis⁷ and gall bladder dysmotility.⁸ In animal models of post operative ileus, an increase in oxidative stress has been linked to the pathogenesis of post operative ileus and treatment with carbon monoxide (CO; antioxidant gas) prevents its development.⁶ In the non obese diabetic (NOD) model of type 1 diabetes, increased oxidative stress has been shown to lead to development of gastroparesis and treatment with antioxidants such as hemin (induces heme oxygenase)⁷ and CO⁹ reverses these changes.

Diabetes represents a state of high oxidative stress as a result of hyperglycemia induced ROS generation. As expected, both in humans¹⁰ and in animal models⁷ there is an increase in antioxidant defenses such as heme oxygenase to counterbalance the high oxidative stress. Gastrointestinal dysmotility can occur in diabetes and patients exhibit symptoms such as nausea, vomiting, bloating, diarrhea or constipation and abdominal pain to a varying degree of severity.¹¹ The etiology of gastrointestinal dysfunction still remains unclear but there has been significant progress in our understanding of the disease pathogenesis. Among other players,¹² oxidative stress appears to be an important player in gastrointestinal complications of diabetes specifically diabetic gastroparesis.⁷ Interstitial cells of Cajal (ICC) and the enteric nervous system appear to be the most significantly affected cell types in

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Disclosures

None

diabetes, though autonomic neuropathy and smooth muscle dysfunction have also been well described and more recently immune cells have been recognized as important players.¹³

In this issue of “Neurogastroenterology and Motility” a team of investigators from Emory University lead by Dr. Srinivasan¹⁴ have helped fill an important gap in our current understanding of the effect of oxidative stress on colonic motility in diabetes. A significant number of diabetic patients suffer from diarrhea or constipation and in the past this was attributed to autonomic neuropathy. However, the group from Emory provides new evidence which suggests loss of enteric neurons in the diabetic colon may also be responsible for these symptoms. A previous study had shown that, in the gastrointestinal tract, colon was more susceptible to damage by oxidative stress,¹⁵ however, there was a lack of clear understanding of how oxidative stress affects the colon. The authors now demonstrate (in 8 patients with diabetes) that there is increased apoptosis of enteric neurons and a decrease in ganglion size in the colon. This was associated with increased oxidative stress as suggested by a decrease in reduced glutathione levels and increase in superoxide dismutase mRNA. Interestingly, inhibitory neuronal subpopulations appear to be more susceptible to the effects of oxidative stress and this is further confirmed by isometric muscle recordings from diabetic colon tissue, which showed a significantly lower increase in contractility in response to nitric oxide inhibitor L-Nitro Arginine Methyl Ester (L-NAME) as compared to controls. Similarly, other studies on the autonomic nervous system have also shown differential susceptibility of sympathetic neurons to oxidative stress.¹⁶ This finding by the group at Emory is consistent with existing data on an impaired sex-dependant nNOS/NO function in nitrergic neurons in the gastrointestinal tract in diabetes.^{17, 18}

The authors further show, in an in-vitro system, using enteric neuronal murine cell lines developed in their laboratory, that the antioxidant lipoic acid activates the P-I-3-kinase signaling pathway and protects neuronal cells from apoptosis in the presence of increased glucose levels. There has been a long standing interest in lipoic acid as an antioxidant for peripheral neuropathy associated with diabetes. Lipoic acid improves nerve blood flow, reduces oxidative stress, and improves distal nerve conduction in experimental diabetic neuropathy.¹⁹ It has been shown to be moderately effective in diabetic peripheral neuropathy²⁰ and based on the current study, it has therapeutic potential in diabetic enteric neuropathy. There are, however, questions that still need to be answered. These include the relative ability of the enteric nervous system to repair itself. Would the best strategy be prevention of damage or targeting those patients with demonstrated loss of neurons? A relevant development in this area has been the demonstration of enteric neurogenesis in adult mice by activation of 5HT4 receptors.²¹ This has the potential for opening new avenues for research and for development of replacement therapy for enteric neuronal loss when coupled to the information we now have from the current study.¹⁴ It is now clear oxidative stress affects multiple cell types in the gastrointestinal tract. Targeting more than one cell type and more specific targeting may therefore be advantageous. Antioxidants such as lipoic acid,²⁰ vitamin E,²² glutathione and melatonin²³ have previously been evaluated in diabetic patients. However, simple oral supplementation of antioxidants may not be enough and we need to develop strategies to stimulate targeted, more robust antioxidant defenses in the tissues of interest. Localized induction of HO1 is one approach.²⁴ Gene therapy using adenovirus mediated HO1 site specific delivery²⁵ and use of products of HO1 such as CO⁹

are other approaches to help shift the balance and protect tissues from ROS mediated damage. Also, the recent demonstration of decrease in intestinal oxidative environment and delayed development of diabetes in Biobreeding rats by administration of the bacterium *L. johnsonii* provides yet another exciting therapeutic avenue.²⁶

Another question which still remains to be answered is if the cellular changes noted in the enteric nervous system correlate with patient symptoms or more objective markers such as colonic transit. In the current study,¹⁴ only 19% diabetic patients had constipation compared to 10% in controls. It would be interesting to see if there were significant differences in the enteric nervous system between symptomatic and asymptomatic patients and if asymptomatic patients had better antioxidant defenses.

Linking symptoms to physiology and to cellular changes are important steps in determining the key targets to go after and rational therapy design. For example, the finding that development of delayed gastric emptying in diabetic NOD mice correlated with loss of ICC rather than loss of nNOS⁷ despite both being reduced, highlights this need. The state of nNOS is also relevant as active nNOS is dimerized¹⁸ as is measurement of bioavailability of nitric oxide.¹⁸ It is often difficult in humans to make such correlations given the difficulty in obtaining tissue and controlling patient and environmental factors. A step towards prospectively acquiring tissue from well characterized patients with gastroparesis has been the establishment of an NIH multicenter consortium recruiting patients with gastroparesis and establishing a tissue database.²⁷ Expansion of this effort to other parts of the gastrointestinal tract, including the colon would be of considerable value.

Another important question which remains to be answered is how can we determine which diabetic patients will develop gastrointestinal complications? There are several mechanisms by which oxidative stress is countered in conditions such as diabetes by upregulating antioxidant defenses. It is when such mechanisms fail that tissue damage results from excess ROS. We need further studies to better identify the vulnerable patient population so they can be treated prior to development of complications. Identifying differences in transcriptional regulation of antioxidant defenses would be one such approach.

In summary, the team of investigators at Emory have shown that in diabetic patients increased oxidative stress in the colon leads to increased apoptosis of enteric neurons specifically the inhibitory neurons and this damage can be prevented, at least in vitro, by lipoic acid. This important finding helps advance the field and opens the door to answer several other important questions which will hopefully lead to the much needed development of newer therapeutic agents for treatment of gastrointestinal complications of diabetes.

Acknowledgments

Supported by NIH grant DK 68055.

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