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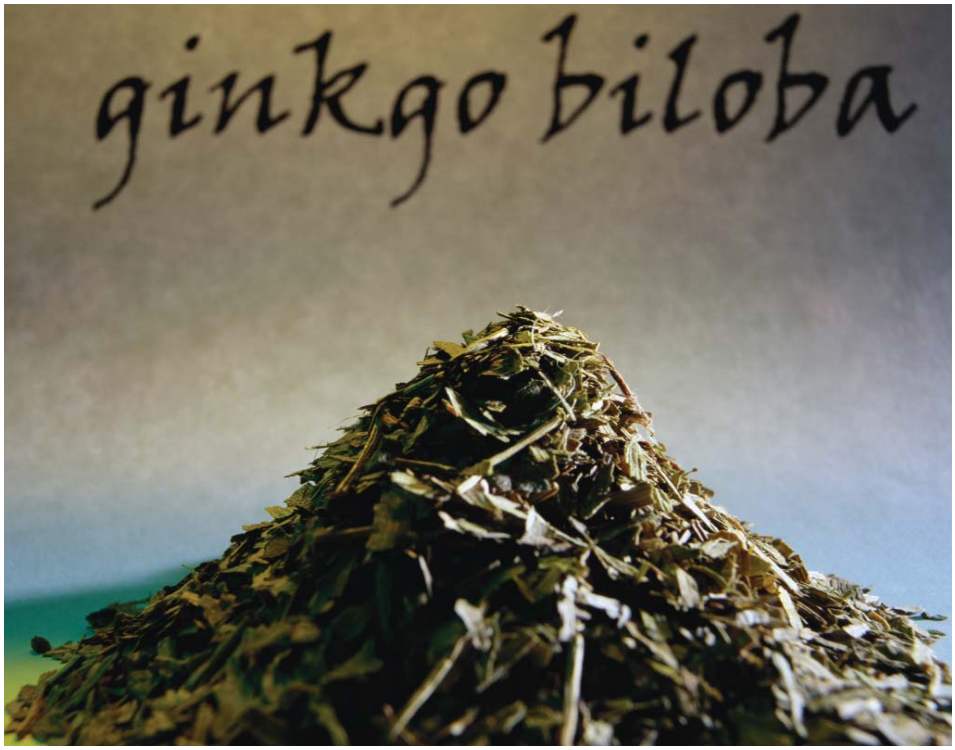
Study backs existing evidence on oxidative stress and IOP-induced damage

Fighting glaucoma with antioxidant treatment

by Matt Young **EyeWorld Contributing Editor**

New research reveals further evidence that antioxidant treatment could help defeat glaucoma. The study, published in the October 2007 issue of *Investigative Ophthalmology & Visual Science*, found that oxidative stress is "an early event in hydrostatic pressure/IOP-induced neuronal damage." It also found that antioxidant treatment reduced oxidative stress in pressure-treated retinal ganglion cells (RGCs).

"These findings support the view that oxidative damage contributes early to glaucomatous optic neuropathy," wrote study co-author **Robert N. Weinreb, M.D.**, Hamilton Glaucoma Center and Department of Ophthalmology, University of California San Diego, La Jolla, Calif. "Our results demonstrate that oxidative damage occurs within hours of elevated hydrostatic pressure or elevated IOP."



Nutritional supplements, such as gingko biloba, could help prevent glaucoma

Making the link

In the study, Dr. Weinreb and colleagues subjected cultured RGC-5 cells to 0, 30, 60, or 100 mm Hg hydrostatic pressure for two hours.

"Immunocytostaining with anti-HNE polyclonal antibody showed that the level of HNE [human neutrophil elastase] adducts was increased greatly in RGC-5 cells exposed to elevated pressure compared with nonpressure control cells," Dr. Weinreb reported. HNE adduct formation is a lipoxidation product.

"HNE immunoreactivity was greatest at 60 mm Hg, with more than a fivefold increase compared with control samples," he noted.

Meanwhile, during a different experiment with HNE, in which the product was administered in different concentrations to RGC-5 cells, Dr. Weinreb found that it indeed caused cell death.

In very small concentrations—5 micromoles—HNE caused significant cell death. At 10 micromoles, it caused 30% cell death within 16 hours, and at 50 micromoles, almost all the cells died in that time frame.

The antioxidant resveratrol had a clear impact on HNE, and quercetin had mixed success.

Pretreatment of RGC-5 cells with 20 or 40 micromoles resveratrol reduced HNE formation induced by pressure by 61% and 60%, respectively. Quercetin had no effect with only 20 micromoles pre-

treatment, but with 40 micromoles pretreatment, pressure-induced HNE was reduced by 29%.

Meanwhile, HO-1 expression (an oxidative stress-induced protein) increased 184% after 2 hours of treatment with 30 mm Hg, and 231% with 60 mm Hg. Oddly though, at 100 mm Hg, the protein level did not change significantly compared with controls.

Nonetheless, the 100 mm Hg level likely did kill cells. "Immediately after the pressure treatment, calcein AM viability/cytotoxicity assay revealed 2 to 4% of RGC-5 cells were nonviable in 100 mm Hg pressure-treated samples, which is consistent with recent findings of a pressure effect on RGC-5 cells," Dr. Weinreb reported. "There was no evidence of cell death in any of the other treatment groups."

In a second experiment, IOP was increased to 30, 60, or 100 mm Hg in mice for one hour, after which time the retinas were examined, as well as HNE adduct formation and HO-1 expression.

"Immunohistochemistry of retina sections with anti-HNE polyclonal antibody showed that HNE adducts were increased in a pressure-dependent manner," Dr. Weinreb reported. "Immunohistochemistry of optic nerve sections with anti-HNE polyclonal antibody showed that HNE adducts were also increased, especially in the optic nerve head portion. Western blot studies using anti-HNE polyclonal antibody consistently showed that elevated IOP for 1-hour induced pressure-dependent increases in HNE adduct formation in retina samples up to 5-fold to the control samples."

Understanding the results

These results indicated that oxidative stress is a response to elevated IOP, Dr. Weinreb noted.

"Our results provide additional evidence that oxidative damage in response to pressure elevation is an important underlying mechanism of hydrostatic pressure/IOP-induced cellular damage and neuron death," Dr. Weinreb concluded. "The inhibition of oxidative damage by antioxidant treatments indicates that these agents may provide preventive or therapeutic intervention for glaucoma."

John D. Sheppard, M.D., professor of ophthalmology, microbiology and immunology, and clinical director, Thomas R. Lee Center for Ocular Pharmacology, Eastern Virginia Medical School, Norfolk, added that there's "no doubt" that oxidative stress is "an essence of aging."

"There's no doubt it should be a part of glaucoma as well," Dr. Sheppard said. "Glaucoma is a disease of aging."

Fortunately, new methods of neuroprotection are being put into practice to target oxidative stress, Dr. Sheppard said.

"Docs are using a lot more antioxidants and neuroprotectant therapy like ginkgo biloba," Dr. Sheppard said. "Companies like ScienceBased Health, [Carson City, Nev.], have developed [over-the-counter] nutritional products derived from clinical and laboratory peer review studies specifically targeting neuroprotection."

These therapies are no longer relegated to niche nutritional labels. "Allergan [Irvine, Calif.] just finished a landmark trial with hundreds of patients over four years involving neuroprotection from memantine," Dr. Sheppard said.

"A glaucoma specific formulation will be available soon. Memantine competitively blocks N-methyl-D-aspartate receptors in damaged neurons. This inhibits glutamate damage seen in neurodegenerative diseases such as glaucoma, Alzheimer's, neuropathy, and spinal cord injuries," he said.

Physicians still do not know which glaucoma patients would benefit from neuroprotection, but certain subsets potentially have more to gain than lose.

"Patients with low tension glaucoma would likely benefit from neuroprotection," Dr. Sheppard said. "IOP is not as much of a factor in these patients as is the susceptibility of the optic nerve head."

Editors' note: Dr. Weinreb has no financial interests related to this study. Dr. Sheppard has no financial interests related to his comments.

Contact Information

Sheppard: 757-622-2200, docshep@hotmail.com
Weinreb: weinreb@eyecenter.ucsd.edu