PHARMACEUTICAL INTERVENTIONS FOR HEARING LOSS (PIHL)

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Biomarkers of Oxidative Damage and Inflammation: Experiences in Hearing and Balance Disorders

Gerald M. Haase MD, Kedar N. Prasad PhD

Oxidative stress from excessive production of free radicals is recognized as a causative factor in noise-induced hearing disorders (Henderson, Bielefeld, Harris, & Hu 2006; Neri et al., 2006; Ohlemiller, Wright, & Dugan, 1999). Pro-inflammatory cytokines from acute and chronic inflammation are also implicated in auditory injury (Chen et al., 2007; Derebery, 1996). Therefore, it is not surprising that several groups of investigators have reported that antioxidants (that also produce anti-inflammatory effects and quench free radicals at appropriate doses) demonstrate benefits in animal models and human trials (Ewert et al.,2012; Haase, Prasad, Cole, Baggett-Strehlau, & Wyatt, 2011; Hatano, Uramoto, Okabe, Furukawa, & Ito, 2008; Kopke et al., 2005; Le Prell, Hughes, & Miller, 2007).

A logical derivative from these experiences would suggest that biomarkers of oxidative damage and inflammation have value as appropriate secondary outcomes parameters in clinical studies regarding interventions for hearing disorders. While the list of potential biomarkers is extensive, it seems reasonable to highlight some of the most clinically relevant and well-studied in each category, including those with which we have had direct positive experience.

Oxidative stress involves direct injury to lipids, DNA and proteins, each of which is related to different biomarkers of damage. Markers of inflammation and antioxidant levels also reflect the degree of injury. The following measures are recommended as an initial relevant panel:

Markers of Oxidative Stress

In order to obtain a reliable indication of oxidative damage, at least one representative from each category of lipid peroxidation, adducts of DNA and oxidation of proteins, should be determined. Published studies that have only measured one or even two biomarkers of the same molecule may provide inadequate information.

Malondialdehyde (MDA) is an intracellular aldehyde formed during the metabolism of polyunsaturated fatty acids and a product of prostaglandin biosynthesis. It is one of the most abundant biomarkers of lipid peroxidation, a process resulting from increased cellular oxidative stress (Feng, Hu, Marnett, & Tang, 2006). One specific assay is based on the reaction of a chromogenic agent with MDA and the spectral absorbance is read at 586nm. The analysis can be performed on both plasma and urine in prospective human trials (Coppes et al., 2006).

F2-isoprostane, resulting from the non-enzymatic free radical-catalyzed peroxidation of arachidonic acid, is considered by some investigators to be a more specific product of lipid peroxidation and a more sensitive indicator of oxidative damage in vivo (Montuschi , Barnes, & Roberts, 2004). Measurements based on gas chromatography, mass spectrometry and immunoassays are available for biological fluids and tissues. Plasma levels can be determined, but because of ease of collection and stability, measures of urinary levels are frequently utilized, such as in randomized human trials involving military subjects (Hodgdon et al., 2008).

Endogenous damage to nuclear or mitochondrial DNA can be caused by oxidative stress. One of the predominant oxidative lesions and most widely detected DNA adducts is 8-hydroxy deoxyguanosine (Marnett ,1999,2002). Detection in urine is commonly available employing mass spectrometry and immunochemical techniques and has been a beneficial marker in clinical trials evaluating oxidative stress occurring in harsh military environments (Hodgdon et al., 2008.).

Nitrosylative stress causes post-translational protein modifications and occurs with increased production of nitric oxide which is oxidized to form peroxynitrite. Peroxynitrite ca uses nitration of tyrosine and forms 3-nitrotyrosine (Ceriello, 2002; Xiao, Nel, & Loo, 2005). Nitrotyrosine in plasma can be detected by mass spectrometry, immune-blotting and gel electrophoresis. Similarly, oxidation of amino acid residues on proteins is a sensitive indicator of cellular damage. The modifications result in the formation of protein carbonyls (Chevion, Berenshtein, & Stadtman , 2000). These substances react with dinitrophenylhydrazine and the subsequent bound product can be measured in plasma by enzyme-linked immunosorbent assay technology.

Biomarkers of Inflammation

Pro- and anti-inflammatory cytokines are released by immune cells during the response to injury. During the acute phase, anti-inflammatory cytokines dominate to assist in repair, whereas pro-inflammatory cytokines are predominant in the chronic phase. An Increase in the level of two such substances, tumor necrosis factor-alpha and interleukin-6, participates in the pathologic processes of many chronic diseases,

including hearing disorders (Clark, 2007; Heinrich et al., 2003). In addition, C-reactive protein is a well-established biomarker of inflammation, infection and tissue damage with relevant predictive clinical value (Pepys, & Hirschfield, 2003). Specific analysis of these substances in plasma can be performed by solid-phase enzyme-linked immunosorbent assay.

Nitric oxide (NO) synthases are enzymes that catalyze production of NO from L-arginine. One form, inducible nitric oxide synthase (iNOS), is of particular value because it is only expressed after cellular activation and produces the most sustained periods of NO presence. iNOS is expressed in a parallel manner in inflammatory processes in humans and rodents making it a valuable experimental biomarker of damage in animal models (Chavko et al., 2008). It is usually measured in plasma samples.

Levels of Glutamate

The excitatory amino acid, glutamate, plays a significant role in the progression of traumatic brain injury and related conditions such as hearing and balance dysfunction (Gopinath, Valadka , Goodman, & Robertson, 2004). Since increased levels of glutamate are detected with increased severity of injury, these measurements in plasma should be determined. The most accurate technical methods employ high performance liquid chromatography.

Antioxidant levels

The degree of oxidative damage can also be determined by antioxidant levels in plasma and the level of alpha-tocopherol (lipid soluble) has proved to be a credible marker. These determinations have been valuable in demonstrating restoration of critical antioxidant levels as well as documenting subject compliance in consuming the study medication (Hodgdon et al., 2008). Glutathione (water soluble) is the most abundant intracellular antioxidant and is present in millimolar rather than micromolar amounts. However, some investigators have demonstrated that under certain circumstances, oxidized glutathione can also promote oxidative processes (Pompella, Visvikis, Paolicchi, & Casini, 2003). Therefore, it is generally thought that the ratio of reduced to oxidized glutathione is the most predictive measurement of the severity of injury (Pastore et al., 2001). A reliable assay employs a thiol-scavenging agent that reduces total glutathione without interfering with the action of glutathione reductase. The change in absorbance of the detectable product is determined by high performance liquid chromatography.

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