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Dietary vitamin C supplementation reduces noise-induced hearing loss in guinea pigs

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Abstract

Vitamin C (ascorbate) is a water-soluble, low molecular weight antioxidant that works in conjunction with glutathione and other cellular antioxidants, and is effective against a variety of reactive oxygen species, including superoxide and hydroxyl radicals that have been implicated in the etiology of noise-induced hearing loss (NIHL). Whereas most animals can manufacture their own vitamin C, humans and a few other mammals such as guinea pigs lack the terminal enzyme for vitamin C synthesis and must obtain it from dietary sources. To determine if susceptibility to NIHL could be influenced by manipulating dietary levels of vitamin C, albino guinea pigs were raised for 35 days on a diet with normal, supplemented or deficient levels of ascorbate, then exposed to 4 kHz octave band noise at 114 dB SPL for 6 h to induce permanent threshold shifts (PTS) of the scalp-recorded auditory brainstem response. Animals that received the highest levels of dietary ascorbate developed significantly less PTS for click stimuli and 4, 8, 12, and 16 kHz tones than animals on normal and deficient diets. Outer hair cell loss was minimal in all groups after noise exposure, but permanent damage to stereocilia were observed in noise-exposed ears. The results support the hypothesis that dietary factors influence individual susceptibility to hearing loss, and suggest that high levels of vitamin C may be beneficial in reducing susceptibility to NIHL.
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Keywords: Antioxidants; Ascorbate; Ascorbic acid; Acoustic trauma; Reactive oxygen species; Free radicals

1. Introduction

Hearing loss is a major health condition affecting more than 28 million Americans. More than 30% of these individuals have hearing loss attributable, at least in part, to noise exposure, and 30 million Americans are exposed to damaging levels of noise on a daily basis (National Institute on Deafness and Other Communicative Disorders, 2002). The most direct ways of preventing noise-induced hearing loss (NIHL) are to avoid exposure to potentially damaging sounds and to utilize appropriate hearing protection devices when acoustic overexposure cannot be avoided. Given the prevalence and impact of NIHL, it is important to explore practical methods for reducing individual susceptibility for those instances when damaging sounds cannot be avoided and hearing protection is either unavailable or impractical to use.

Recent studies using experimental animals have focused on antioxidant administration as a means of decreasing susceptibility to NIHL. Antioxidants prevent cochlear damage from toxic reactive oxygen species (ROS) that are produced at high levels during and after noise exposure (Ohlemiller et al., 1999; Yamane et al., 1995a,b). Glutathione (GSH), a small, water-soluble thiol with potent antioxidant activity (Lomaestro and

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Malone, 1995; Meister, 1982; Meister et al., 1986), has proven effective in attenuating NIHL in several animal models and noise exposure paradigms. Because GSH is not readily transported across cell membranes, it has been necessary to supply substrates, GSH derivatives, or GSH-promoting agents to increase GSH levels in the inner ear. Experimental approaches that have proven effective in increasing cochlear GSH levels and reducing subsequent NIHL include administration of glutathione monoethyl ester (Henderson et al., 1999b; Hight et al., 2003; Ohinata et al., 2000a), *N*-L-acetylcysteine (Duan et al., 2004), *R*-phenylisopropyladenosine (*R*-PIA) (Hight et al., 2003; Hu et al., 1997), and 2-oxothiazolidine-4-carboxylate (Yamasoba et al., 1998). Administration of other antioxidants, such as ebselen (Lynch et al., 2004; Pourbakht and Yamasoba, 2003a) and superoxide dismutase (Seidman et al., 1993), have also been effective in reducing temporary threshold shifts (TTS) and more importantly, permanent threshold shifts (PTS) caused by acoustic over stimulation. The effectiveness of various antioxidant treatments varies considerably according to drug dose and timing of administration relative to the noise exposure, due to antioxidant kinetics and the mechanisms of ROS generation, activity and deactivation (Duan et al., 2004; Henderson et al., 1999a,b; Hight et al., 2003; Hu et al., 2001).

Although most experimental approaches to increasing antioxidant levels have proven effective in reducing the magnitude of NIHL, they all share drawbacks with respect to route of drug administration and timing of multiple doses relative to noise exposure. In most previous studies, antioxidants or antioxidant-promoting drugs were administered one or more times before and/or after noise exposure via systemic injections (Duan et al., 2004; Lynch et al., 2004; Ohinata et al., 2000a; Seidman et al., 1993; Yamasoba et al., 1998) or by application of the drug to the round window membrane (Hight et al., 2003; Hu et al., 1997, 2001). To be of practical value, the antioxidant treatments must be easy to administer and antioxidants must be available in the cochlea at appropriate times relative to ROS generation. Dietary supplementation of antioxidants or their precursors may provide a means of overcoming limitations regarding route and timing of antioxidant administration. Normal diets include a wide range of compounds with antioxidant properties, including vitamins C (ascorbate), E (α-tocopherol), and A (retinol), and diverse polyphenols, including flavinoids and organosulfur compounds (Esposito et al., 2002; Jovanovic and Simic, 2000; Quiles et al., 2002). It is reasonable to hypothesize that variations in dietary intake of antioxidants may influence individual susceptibility to hearing loss, and that enrichment of antioxidants through dietary intake may provide significant protection from NIHL.

In the current study, we investigated the effects of dietary vitamin C levels on susceptibility to PTS in albino guinea pigs. Like GSH, vitamin C is a small, water-soluble, broad-spectrum antioxidant (Chatterjee, 1978; Ghosh et al., 1996; Meister, 1992; Rose and Bode, 1993; Som et al., 1983). Unlike GSH, vitamin C is transported via the bloodstream to all organs, including the brain, and across cell membranes by sodium-dependent ascorbate transporters, where it accumulates in tissues (Daruwala et al., 1999; Goldenberg and Schweinzer, 1994). Vitamin C regenerates α-tocopherol, spares GSH, offsets GSH deficiency in cells, and works synergistically with GSH and other antioxidants (Jain et al., 1992; Meister, 1992; Rose and Bode, 1993). Based on the cellular ubiquity and antioxidant properties of vitamin C, we hypothesized that animals fed a vitamin C-deficient diet would develop more PTS than control animals on a normal diet, whereas animals fed a vitamin C-enriched diet would develop less PTS than controls.

2. Methods

2.1. Subjects and diets

Twenty-four outbred Dunkin Hartley albino guinea pigs (HsdPoc:DH strain) were obtained from Harlan (Indianapolis, IN). The guinea pigs were two weeks old and weighed 205–269 g at the beginning of the study. Animals were randomly assigned to three diet groups, Supplemented, Normal and Deficient, with four males and four females in each group. Animals were housed in separate cages and maintained on a 12:12 h light:dark cycle in a single room where ambient noise levels were below 40 dB SPL at frequencies above 1 kHz. All procedures regarding the care and use of the guinea pigs were reviewed and approved by the Institutional Animal Care and Use Committee at UB (Project Number HER07043N).

Three custom guinea pig pellet diets, differing only in vitamin C levels, were obtained from Harlan-Teklad (Madison, WI). Harlan-Teklad modified their basic vitamin C-deficient guinea pig diet (TD 93061) by adding L-ascorbyl-2-polyphosphate (“Stay-C 35”) at levels of 50, 500, and 5000 mg/kg food to create “deficient”, “normal”, and “supplemented” diets, respectively. Because vitamin C is innately unstable and can be readily oxidized when exposed to oxygen or certain minerals, stabilized forms of vitamin C such as L-ascorbyl-2-polyphosphate are recommended over L-ascorbic acid for animal diets (de Rodas et al., 1998). Harlan-Teklad has used L-ascorbyl-2-polyphosphate instead of coated ascorbic acid in their standard primate and guinea pig diets for several years, because of its increased stability during the manufacturing process and prolonged shelf life under various storage conditions (Barb Mickelson,

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