Hearing Research 304 (2013) 33–40

Contents lists available at SciVerse ScienceDirect

Hearing Research

journal homepage: www.elsevier.com/locate/heares

Research paper

Prophylactic and therapeutic functions of drug combinations against noise-induced hearing loss

Jianxin Bao [a,*], Michelle Hungerford [a], Randi Luxmore [a], Dalian Ding [b], Ziyu Qiu [a], Debin Lei [a], Aizhen Yang [a], Ruqiang Liang [a], Kevin K. Ohlemiller [a]

[a] Department of Otolaryngology, Center for Aging, Washington University School of Medicine, 4560 Clayton Avenue, St. Louis, MO 63110, USA
[b] Center for Hearing & Deafness, State University of New York at Buffalo, 137 Cary Hall, 3435 Main Street, Buffalo, NY 14214, USA

ARTICLE INFO

Article history:
Received 7 March 2013
Received in revised form
31 May 2013
Accepted 10 June 2013
Available online 18 June 2013

ABSTRACT

Noise is the most common occupational and environmental hazard. Noise-induced hearing loss (NIHL) is the second most common form of sensorineural hearing deficit, after age-related hearing loss (presbycusis). Although promising approaches have been identified for reducing NIHL, currently there are no effective medications to prevent NIHL. Development of an efficacious treatment has been hampered by the complex array of cellular and molecular pathways involved in NIHL. We turned this difficulty into an advantage by asking whether NIHL could be effectively prevented by targeting multiple signaling pathways with a combination of drugs already approved by U.S. Food and Drug Administration (FDA). We previously found that antiepileptic drugs blocking T-type calcium channels had both prophylactic and therapeutic effects for NIHL. NIHL can also be reduced by an up-regulation of glucocorticoid (GC) signaling pathways. Based on these findings, we tested a combination therapy for NIHL that included ethosuximide and zonisamide (anticonvulsants) and dexamethasone and methylprednisolone (synthetic GCs) in mice under exposure conditions typically associated with dramatic permanent threshold shifts (PTS). We first examined possible prophylactic effects for each drug when administered alone 2 h before noise, and calculated the median effective dose (ED_{50}). We then tested for synergistic effects of two-drug combinations (anticonvulsant + GC), and identified combinations with the strongest synergy against NIHL, based on a previously established combination index (CI) metric. We repeated similar tests to determine their therapeutic effects when administered the same drugs 24 h after the noise exposure. Our study shows the feasibility of developing pharmacological intervention in multiple pathways, and discovering drug combinations with optimal synergistic effects in preventing permanent NIHL.

1. Introduction

Noise-induced hearing loss (NIHL) is the single predominant health hazard posed by occupational and recreational settings (Bohnker et al., 2002; Henderson et al., 2003; Seixas et al., 2005). Although promising approaches have been identified for reducing NIHL mainly based on free radical pathways (Campbell et al., 2007; Kopke et al., 2005; Le Prell and Bao, 2012; Lynch and Kil, 2005), currently no effective pharmacologic agents are approved by FDA to diminish permanent threshold shifts (PTS). Development of an efficacious treatment has been hampered by the complex array of cellular and molecular pathways involved in NIHL.

One major mechanism underlying NIHL is mitochondrial free radical formation due to noise-induced intense metabolic activity in the cochlea (Darrat et al., 2007; Henderson et al., 2006; Le Prell et al., 2003). The involvement of this pathway in NIHL is strongly supported by three main lines of evidence: (1) Noise-induced increase of free radicals is observed in stria vascularis, outer hair cells (OHCs), supporting cells of the organ of Corti, and spiral ganglion (Ohinata et al., 2000; Ohlemiller, 2006; Yamane et al., 1995). This elevation may continue up to at least 14 days post-exposure (Yamashita et al., 2004); (2) Depletion of endogenous antioxidants such as superoxide dismutase and glutathione peroxidase results in increased susceptibility to NIHL (McFadden et al., 2001; Ohlemiller et al., 1999, 2000); (3) Enhancement of antioxidants attenuates NIHL (Lynch et al., 2004; McFadden et al., 2005; Ohinata et al., 2003). Thus, it is not surprising that attempts to prevent NIHL with antioxidants have become the focus of much research (Bielefeld et al., 2007; Hight et al., 2003; McFadden et al., 2005;

* Corresponding author. Tel.: +1 314 747 7199; fax: +1 314 747 7230.
E-mail address: jbao@wustl.edu (J. Bao).

0378-5955/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.heares.2013.06.004

Seidman et al., 1993; Yamashita et al., 2005). Nevertheless, most interventions with single agents are only partially effective in preventing NIHL. A few studies have sought to intervene at multiple sites in free radical pathways, or in combination with other pathways. Synergic effects have been observed in some, but not all, studies (Le Prell et al., 2007; Yamasoba et al., 1999).

Two additional pathways that have emerged as major contributors to NIHL involve calcium and glucocorticoid (GC) signaling. Disturbance in calcium homeostasis has long been suspected to contribute to trauma-induced neuronal injury (Hansen et al., 2003; Nikonenko et al., 2005; Park et al., 2008; Werling et al., 2007). Calcium homeostasis in the cochlea is maintained in part by several types of calcium channels, which include voltage-gated calcium channels (VGCCs) (Adamson et al., 2002; Errington et al., 2005; Fuchs, 2002; Rodriguez-Contreras and Yamoah, 2001; Schnee and Ricci, 2003). VGCCs can be divided into two groups: high-voltage-activated (L-type) and low-voltage-activated calcium channels (T-type) (Lacinova et al., 2000; Perez-Reyes, 1998; Triggle, 2006; Yunker and McEnery, 2003). Blockers of L-type channels have been found to attenuate NIHL in some studies (Heinrich et al., 1997; Uemaetomari et al., 2009), but not others (Boettcher, 1996; Boettcher et al., 1998; Ison et al., 1997). We reported that NIHL can be reduced by the administration of anticonvulsant drugs blocking T-type calcium channels, applied either before or after noise exposure, and these channels are present in the organ of Corti and spiral ganglion neurons (SGNs) (Shen et al., 2007). Inhibition of T-type calcium channels also protects neurons after stroke (Nikonenko et al., 2005). Thus, it is possible that anticonvulsant drugs blocking T-type calcium channels can prevent injury-induced alterations of calcium homeostasis that contribute to NIHL.

Another major molecular mechanism in NIHL involves glucocorticoid signaling. Synthetic GCs are already used clinically to treat hearing loss in a variety of cochlear disorders such as autoimmune inner ear disease, tinnitus and Meniére's disease (Dodson et al., 2004; MacArthur et al., 2008; Trune and Canlon, 2012). Extensive evidence also suggests an important role of GC pathways in NIHL. First, glucocorticoid receptors (GRs) are present in the cells of the organ of Corti, spiral ligament, spiral limbus, and SGNs (Shimazaki et al., 2002; ten Cate et al., 1993; Zuo et al., 1995). Second, stressful 'preconditioning' events such as restraint, heat stress, and even low-level sound—all of which are likely to engage GC signaling—have been found to be protective against NIHL in animals (Paz et al., 2004; Wang and Liberman, 2002; Yoshida et al., 1999). Third, because the noise exposure itself is a stressful event, pretreatment with blockers of GC signaling accordingly makes animals more susceptible to NIHL (Tahera et al., 2006a). Fourth, synthetic GCs such as dexamethasone and methylprednisolone can protect against NIHL (Canlon et al., 2007; Sendowski et al., 2006; Tabuchi et al., 2006; Tahera et al., 2006b, 2006c). Fifth, although GCs can bind to both GR and mineralocorticoid receptors, antagonists against mineralocorticoid receptors have no effect on NIHL (Tahera et al., 2006a). Finally, a series of studies have systematically revealed the role of GR signaling pathways in NIHL (Canlon et al., 2007; Lang et al., 2006; Peppi et al., 2011; Tahera et al., 2006b, 2006c; Shen et al., 2011).

Interventions based on synthetic GC drugs or anticonvulsants blocking T-type calcium channels show limited success in preventing NIHL (Canlon et al., 2007; Shen et al., 2007). Since these agents are from completely different drug families, and likely act on different molecular pathways underlying NIHL, identifying optimal combinations of these that may act in a synergistic manner seemed to us a logical next step. Here we describe methods for identifying and quantifying synergistic drug interactions against NIHL in a mouse model.

2. Materials and methods

2.1. Animals

All animal procedures were approved by the Animal Studies Committee at Washington University in St. Louis. The study included a total 297 C57BL/6J mice aged two months (150 males and 147 females), purchased from The Jackson Laboratory (Bar Harbor, ME, USA). All mice were housed three to five per cage in a noise-controlled environment on a 12 h light/dark cycle with light onset at 6:00 a.m. Except as noted (Fig. 5) all experimental groups contained equal numbers of male and female animals.

2.2. Drug application

Animals were subject to one of two protocols, a 'prevention' protocol under which drugs were administered 2 h prior to a single noise exposure, and a 'treatment' protocol wherein drugs were administered 24 h after noise. Two drugs from each family were chosen based on their favorable clinical usage and low side effects. All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). The dosage range for each drug was decided in two steps. We began with the known median effective dose (ED_{50}) from previous studies. We then expanded the dosage around the ED_{50} by adding at least two concentrations to determine the ED50 against NIHL. Each drug was dissolved in either physiological saline solution (ethosuximide and zonisamide) or vegetable oil (dexamethasone and methylprednisolone), and then administered intraperitoneally. Control animals were injected with physiological saline solution or vegetable oil. The groups exploring drug combinations received two injections (one for each drug). Since one of our main research goals was to reduce side-effects of these drugs by reducing their dosages, we focused on possible synergies against NIHL from each drug's ED_5 to ED_{20}.

2.3. Noise exposure

All animals were exposed the noise at two months old. As described previously (e.g., Bao et al., 2004), noise exposures were performed in a foam-lined, double-walled soundproof room (Industrial Acoustics). The noise exposure apparatus consisted of a 21 × 21 × 11 cm wire cage mounted on a pedestal inserted into turntable. The cage was rotated at 1 revolution/80 s. A Motorola KSN1020A piezo ceramic speaker (four totals) was attached to each side of a metal frame surrounding the cage. Opposing speakers were driven by independent channels of a Crown D150A power amplifier. Noise was generated by two General Radio 1310 generators and filtered to 4.0–45.0 kHz by Krohn-Hite 3550 filters. The overall noise level was measured at the center of the cage using a B&K 4135 ¼ inch microphone in a combination with a B&K 2231 sound level meter set to broadband (0.2–70 kHz). Mice were exposed one time in pairs at 110 dB sound pressure level (SPL) for 30 min.

2.4. Auditory brainstem recording (ABR)

ABR testing was performed prior to treatment, then two weeks after the noise exposure to estimate PTS. ABR thresholds were obtained as described previously (Ohlemiller et al., 2000a,b; Bao et al., 2005). For ABR recording, we used Tucker-Davis Technologies (TDT) System II hardware and software. Animals were anesthetized with a solution of ketamine and xylazine (80/15 mg/kg, i.p.) and positioned dorsally in a custom headholder. Subdermal platinum needle electrodes (Grass) were placed in the mid-back (ground), behind the right pinna (active), and at the vertex