Brain Research 1646 (2016) 467–474

Contents lists available at ScienceDirect

Brain Research

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

Research report

Photobiomodulation rescues the cochlea from noise-induced hearing loss via upregulating nuclear factor κB expression in rats

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ARTICLE INFO

Article history:
Received 20 April 2016
Received in revised form
16 June 2016
Accepted 20 June 2016
Available online 21 June 2016

Keywords:
Photobiomodulation
Noise-induced hearing loss
Nuclear factor κB
Inducible nitric oxide synthase
Cleaved caspase-3

ABSTRACT

Photobiomodulation (PBM) is a noninvasive treatment that can be neuroprotective, although the underlying mechanisms remain unclear. In the present study, we assessed the mechanism of PBM as a novel treatment for noise-induced hearing loss, focusing on the nuclear factor (NF)-κB signaling pathway. Sprague–Dawley rats were exposed to 1-octave band noise centered at 4 kHz for 5 h (121 dB). After noise exposure, their right ears were irradiated with an 808 nm diode laser beam at an output power density of 165 mW/cm^2 for 30 min a day for 5 consecutive days. Measurement of the auditory brainstem response revealed an accelerated recovery of auditory function in the groups treated with PBM compared with the non-treatment group at 4, 7, and 14 days after noise exposure. Immunofluorescent image analysis for inducible nitric oxide synthase and cleaved caspase-3 showed lesser immunoreactivities in outer hair cells in the PBM group compared with the non-treatment group. However, immunofluorescent image analysis for NF-κB, an upstream protein of inducible nitric oxide synthase, revealed greater activation in the PBM group compared with the naïve and non-treatment groups. Western blot analysis for NF-κB also showed stronger activation in the cochlear tissues in the PBM group compared with the naïve and non-treatment groups ($p < 0.01$, each). These data suggest that PBM activates NF-κB to induce protection against inducible nitric oxide synthase-triggered oxidative stress and caspase-3-mediated apoptosis that occur following noise-induced hearing loss.

1. Introduction

Noise-induced hearing loss (NIHL) is a major source of hearing disability in adults worldwide (Nelson et al., 2005). At the cellular level, noise-induced cochlear damage consists of metabolic disruption, which includes ischemia (Nuttall, 1999), excitotoxic damage (Puel et al., 1998), and metabolic exhaustion (Chen et al., 2000). Hearing loss caused by noise damage to auditory hair cells is normally irreversible, as mammalian hair cells do not regenerate. Once activated, this metabolic disruption results in permanent hair cell death, which can occur via apoptosis (Nicotera et al., 2003).

Photobiomodulation (PBM), also referred to as low-level laser therapy, has previously been applied as a noninvasive treatment for promoting cell regeneration and repair (Huang et al., 2009; Tata and Waynant, 2011). The US Food and Drug Administration has approved PBM for the treatment of wound healing, chronic pain, musculoskeletal complications, and other diseases (Conlan et al., 1996; Streeter et al., 2004). The power of low-level lasers varies from 10 to 1000 mW/cm^2 in the continuous mode. The wavelengths used for treatment extend from the visible ($\lambda = 400$ nm) to the near-infrared ($\lambda = 1000$ nm) ends of the spectrum.

The exact protective mechanisms of PBM remain unclear. Recently, we reported that following noise overstimulation, PBM can: (1) accelerate recovery of auditory function; (2) attenuate loss of outer hair cells (OHCs), which function as acoustic pre-amplifiers; (3) inhibit inducible nitric oxide synthase (iNOS), which produces large amounts of neurotoxic reactive oxygen species (ROS) and reactive nitrogen species (RNS); and (4) suppress the activation of

Abbreviations: ABR, auditory brainstem response; iNOS, inducible nitric oxide synthase; PBM, photobiomodulation; NF-κB, nuclear factor kappa B; NIHL, noise-induced hearing loss; OHC, outer hair cell; p-Akt, phospho-Akt; ROS, reactive oxygen species; RNS, reactive nitrogen species; SGC, spiral ganglion cell

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http://dx.doi.org/10.1016/j.brainres.2016.06.031
0006-8993/

caspase-3, the major apoptotic effector protease (Tamura et al., 2015). Recent studies have suggested that PBM affects the activation of nuclear factor (NF)-κB transcription factor (Assis et al., 2012; Rizzi et al., 2006), which plays an important role in iNOS expression after noise exposure (Masuda et al., 2006; Yamamoto et al., 2009). However, the exact role of NF-κB in the cochlea following noise exposure, and the changes in NF-κB after PBM for the treatment of acoustic trauma, are unknown. Based on previous reports (Assis et al., 2012; Rizzi et al., 2006), we hypothesized that PBM modulates the activation of NF-κB after noise overstimulation. Thus, in the present study, we investigated the effects of PBM on the NF-κB signaling pathway in a rat model of NIHL.

2. Results

2.1. Auditory brainstem response threshold shift

Immediately after noise exposure (day 0), the threshold shifts were found in non-treatment and PBM groups (Fig. 1). At day 4, the threshold shift was significantly lower in the PBM group compared with the non-treatment group at 12, 16, and 20 kHz ($p < 0.01$ for each) (Fig. 1). At day 7, PBM groups showed a decreased threshold shift compared with the non-treatment group at 12 kHz ($p < 0.05$) and at 16 and 20 kHz ($p < 0.01$ for each) (Fig. 1). At day 14, PBM groups showed a decreased threshold shift compared with the non-treatment group at 16 ($p < 0.01$) and 20 kHz ($p < 0.05$) (Fig. 1). Statistically significant differences in threshold shift were not found at day 28 (Fig. 1). These results indicate that PBM accelerates recovery of auditory function.

2.2. Immunofluorescent image analysis for iNOS

To investigate the changes of expression of iNOS after noise exposure, we performed immunofluorescent image analysis of OHCs in all groups. At 1 h after noise exposure, we observed strong immunoreactivity for iNOS in the basal, middle and apical turns of OHCs in the surface preparations (non-treatment group), whereas less immunoreactivity was observed in the PBM group (Fig. 2). This indicates that PBM attenuates expression of iNOS at 1 h after noise exposure.

2.3. Immunofluorescent image analysis for cleaved caspase-3

To investigate the changes of expression of cleaved caspase-3 after noise exposure, we performed immunofluorescent image analysis of OHCs in all groups. At 8 h after noise exposure, we observed strong immunoreactivity for cleaved caspase-3 in the basal, middle and apical parts of OHCs in the surface preparations (non-treatment group), whereas less immunoreactivity was observed in the PBM group (Fig. 3). These results indicate that PBM attenuates expression of cleaved caspase-3 at 8 h after noise exposure.

2.4. Immunofluorescent image analysis for NF-κB

In the organs of Corti, we observed weak NF-κB immunoreactivity in the basal turn of OHCs in the non-treatment and PBM groups; no obvious differences were observed between the groups (Fig. 4). In the basal turn of the fibrocytes of the lateral wall and spiral ganglion cells (SGCs), stronger immunoreactivity for NF-κB was observed at 1 h after noise exposure in the PBM group compared with the non-treatment group (Fig. 4).

Subsequently, we quantified the numbers of NF-κB-positive

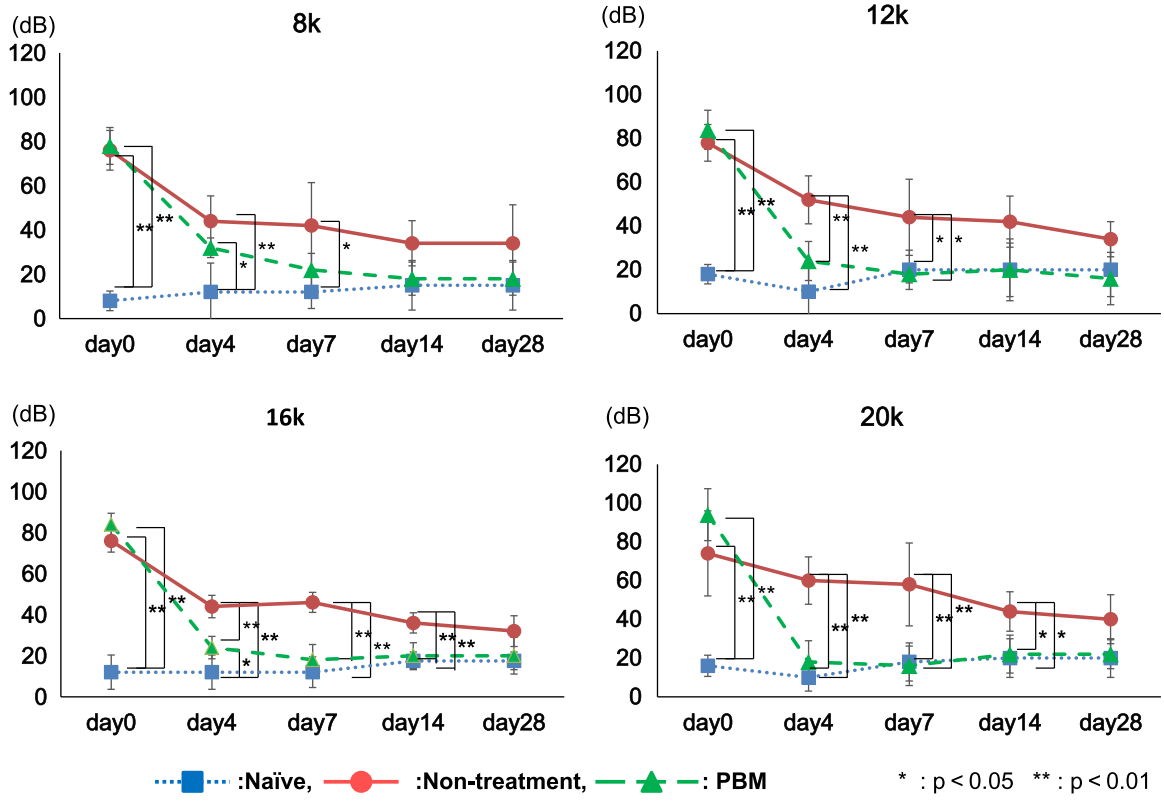

Fig. 1. Auditory brainstem response threshold shift is attenuated by photobiomodulation (PBM). Naïve (blue), Non-treatment (red), and PBM (green). PBM attenuates the noise-induced threshold shift. In the PBM group, PBM significantly attenuated the noise-induced threshold shift at 12, 16, and 20 kHz at days 4 and 7. The values represent the mean ± SD. *$p < 0.05$.