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Research Report

Administration of amitriptyline attenuates noise-induced hearing loss via glial cell line-derived neurotrophic factor (GDNF) induction

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ABSTRACT

Antidepressant treatments have been described to induce neurotrophic factors (NTFs) and reverse the cell loss observed in rodent stress models. Amitriptyline (AT), a tricyclic antidepressant agent, has been reported in recent studies to induce glial cell line-derived neurotrophic factor (GDNF) synthesis and release in rat C6 glioblastoma cells. GDNF has shown protection against acoustic trauma in previous studies. Therefore, we investigated whether AT could induce GDNF synthesis in the cochlea and attenuate cochlea damage against acoustic trauma. We used Hartley guinea pigs and injected AT (30 mg/kg) or saline into the peritoneum. Subjects were exposed to 117 dB SPL octave band noise centered at 4 kHz for 24 h. Noise-induced hearing loss (NIHL) was assessed with auditory brain stem response (ABR) at 4, 8 and 16 kHz measured prior to the injection, 3 days and 7 days after noise exposure. For histological assessment, we observed the sensory epithelium using a surface preparation technique and assessed the quantitative hair cell (HC) damage. We evaluated GDNF synthesis with or without intense noise exposure at 3, 12 and 24 h after the administration of AT in the cochlea using Western blot analysis. GDNF expression was shown 3 h and 12 h after the injection without noise, whereas with noise the GDNF expression lasted for 24 h. The AT-administrated group showed significantly reduced ABR threshold shift and less HC damage than the saline-administrated group. These findings suggest that the administration of AT-induced GDNF levels in the cochlea and attenuated cochlea damage from NIHL.

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Abbreviations: AT, amitriptyline; HC, hair cells; IHCs, inner hair cells; OHCs, outer hair cells; NTFs, neurotrophic factors; NTF, neurotrophic factor; ABR, auditory brainstem response; GDNF, glial cell line-derived neurotrophic factor; BDNF, brain-derived neurotrophic factor; TGF-beta1, transforming growth factor; NT-3, neurotrophin-3; FGF-2, fibroblast growth factor; NMDA, *N*-methyl-D-aspartate; GFR-α, GDNF family receptor α; c-Ret, tyrosine kinase receptor; TTS, temporary threshold shift; PTS, permanent threshold shift; MAPK, mitogen-activated protein kinase; MEK, MAPK-extracellular signal-regulated kinase (ERK) kinase

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1. Introduction

Sensorineural hearing loss results from a variety of causes such as exposure to intense sounds, aging, infection or drugs with ototoxic side effects. Degeneration of cochlear hair cells (HCs) in humans and other mammals is irreversible, resulting in permanent hearing impairment (Tsue et al., 1994). Noise-induced hearing loss (NIHL) is a common cause of sensorineural hearing loss in industrial countries. Among the 120 million people in Japan, an estimated 2.5 million people are exposed to hazardous noise (Yoshiaki et al., 2003). This functional impairment reflects direct acoustic mechanical trauma and/or metabolic damage. Recent studies have described a variety of substances such as dexamethasone, salicylates, trolox, N-acetylcysteine, N-methyl-D-aspartate (NMDA) receptor antagonist and geranylgeranylacetone which protect or rescue auditory function and structure after NIHL (Mikuriya et al., 2005; Ohinata et al., 2003; Takemura et al., 2004; Yamashita et al., 2005).

Recent studies have shown that neurotrophic factors (NTFs) influence the development, growth and survival of neuronal and non-neuronal tissues (Fritzsch et al., 1999; Sariola et al., 2003). In the organ of Corti, NTFs such as brain-derived neurotrophic factor (BDNF), transforming growth factor (TGF-beta1), neurotropin-3 (NT-3) and fibroblast growth factor (FGF-2) have shown protective effects against NIHL and ototoxic trauma (Kawamoto et al., 2003; Low et al., 1996; McGuinness et al., 2005; Shoji et al., 2000a; Yamasoba et al., 1999). Among these NTFs, glial cell line-derived neurotrophic factor (GDNF) has successfully protected spiral ganglion neurons and HCs against various insults such as aminoglycoside ototoxicity and NIHL by chronic infusion or gene transfer into the cochlea (Kawamoto et al., 2003; Keithley et al., 1998; Shoji et al., 2000b; Yagi et al., 1999; Ylikoski et al., 1998). GDNF is a glycosylated, disulfide-bonded homodimer that is distantly related to the transforming growth factor-β (TGF-β) superfamily. It was originally isolated from the rat glial cell line B49 (Lin et al., 1993) and is a potent NTF that enhances the survival of the peripheral and central nervous system (Lindsay et al., 1994; Yan et al., 1995), such as dopaminergic neurons (Cass et al., 1996; Choi-Lundberg et al., 1997), motor neurons (Henderson et al., 1994; Yan et al., 1995) and auditory neurons (Keithley et al., 1998). GDNF has been applied to the inner ear by various methods, such as chronic infusions with an osmotic pump, adenoviral vectors and directly applied to the round window membrane (Kawamoto et al., 2003; Keithley et al., 1998; Shoji et al., 2000b; Yagi et al., 1999, 2000; Yamasoba et al., 1999; Ylikoski et al., 1998). All methods were successful at protecting spiral ganglion neurons and HCs from degeneration, although all application methods of GDNF required a surgical approach. Surgery itself has the possibility of damaging the auditory epithelium; therefore, inducing endogenous GDNF in the inner ear via a systemically administrated agent would be clinically safer approach and ideal for treatment against NIHL.

Initial reports have described that antidepressant treatments induce the expression of NTFs such as BDNF, FGF-2 and GDNF in the hippocampal neurons, cortical neurons and rat astrocytes (Duman et al., 2004; Hisaoka et al., 2001; Mallei et al., 2002). Amitriptyline (AT), a tricyclic antidepressant agent commonly used for the management of depression and chronic pain, has been reported in recent studies to induce GDNF synthesis and release in rat C6 glioblastoma cells (Hisaoka et al., 2001). AT acts on the noradrenergic or both the noradrenergic and serotonergic systems and exerts potent effects to block alpha 2A adrenoreceptor. From these data, we hypothesized that AT may induce GDNF synthesis in the cochlea and acting as a protective factor against NIHL, thus making it possible to increase GDNF levels in the cochlea without a surgical intervention. In this study we aimed to systematically investigate the protective effect of AT via GDNF synthesis against NIHL in an in vivo guinea pig cochlea model.

2. Results

2.1. *Auditory brainstem response thresholds*

To determine the ABR threshold shift of AT-treated and saline-treated subjects after noise exposure we measured ABR 3 and 7 days after noise exposure and compared these values with the baseline ABR threshold (before noise exposure). As shown in Fig. 1, all frequencies (4, 8 and 16 kHz) showed elevated thresholds in both subjects. There was a statistically significant difference at each frequency tested in threshold shift between subjects receiving AT 30 mg/kg injections and subjects receiving an equivalent volume of saline ($p<0.05$). These results showed that the AT injection attenuated the elevation of noise-induced threshold shift.

2.2. *GDNF expression*

To determine if a peritoneal injection of AT could induce GDNF in the cochlea by various time intervals (3 h, 12 h and 24 h), we measured GDNF protein levels in the cochlea using Western blot analysis. The cochlea was homogenized in cell lysis buffer and the amount of protein was determined using the Bradford method (Bradford et al., 1976). One milligram of protein was

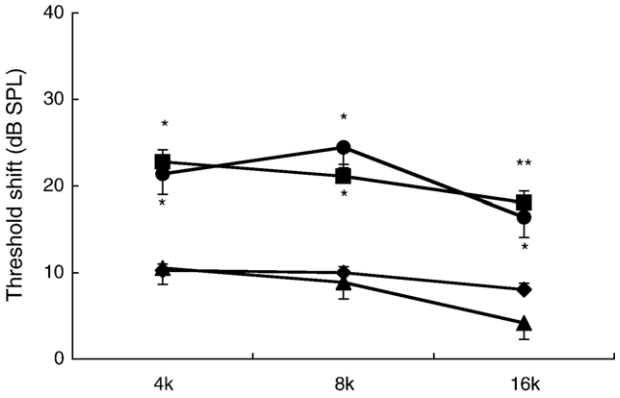

Fig. 1 – The ABR thresholds of AT and saline-treated ears 3 days and 7 days after intense noise exposure. The AT-administrated 3 day (♦, $n=9$) and 7 day (▲, $n=9$) groups showed significantly reduced threshold shifts compared to the saline-treated 3 day (■, $n=9$) and 7 day (●, $n=9$) groups at all frequencies. Error bar±SEM. $^{*}p<0.01$, $^{**}p<0.05$