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Research Report

Activated protein C rescues the cochlea from noise-induced hearing loss

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ABSTRACT

Activated protein C (APC) is a serine/threonine protease and a physiological anticoagulant that exerts anti-inflammatory and anti-apoptotic effects. Although recent studies have revealed that APC has the potential to protect endothelial cells from apoptosis, the mechanisms of its cytoprotective effect are not fully understood. We examined the potential of APC to protect against noise-induced hearing loss (NIHL) and investigated phosphorylation of serine-threonine kinase (Akt) and inhibition of apoptosis as possible cytoprotective mechanisms. We administered intraperitoneal injections of APC (150, 300 U/kg) or normal saline to rats 30 min before exposure to a sound pressure level (SPL) of 126 dB and 4-kHz octave band noise for 5 h. The auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE) were measured before and after exposure. ABR and DPOAE measurements revealed greater improvement in the APC group than in the control group 28 days after exposure. Our examination of outer hair cells (OHCs) at 28 days after noise exposure revealed a significantly higher OHC survival rate in the APC group than in the control group. Immunohistochemical analyses for cleaved-caspase 3, phospho-p38 (p-p38), TUNEL, and phospho-Akt (p-Akt) revealed strong immunoreactivities against cleaved-caspase 3, p-p38, and TUNEL in the inner ear tissues of the control group; however, these signals were decreased in the APC group. Moreover, APC significantly induced activation of p-Akt in the cochlea. These findings suggest that APC has a novel protective effect on the cochlea against NIHL that is mediated by p-Akt and the anti-apoptotic signaling pathway.

1. Introduction

Noise-induced hearing loss (NIHL) is a major source of hearing disability in the adult population 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), metabolic exhaustion (Chen et al., 2000), and cochlear

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fluid intermixing (Bohne and Rabbitt, 1983). Hearing loss due to noise damage to auditory hair cells is normally irreversible because mammalian hair cells do not regenerate. Once activated, this metabolic disruption results in permanent hair cell death, which can occur by the process of apoptosis. Recently, several molecules with antioxidant and scavenging properties have been shown to reduce oxidative stress-induced hair cell death after intense sound exposure (Le Prell et al., 2007). However, success in the laboratory has not translated to the clinic, and neuroprotection-based treatment strategies for sensorineural hearing loss have been disappointing so far.

Activated protein C (APC) is a serine protease with systemic anticoagulant activity and direct cellular effects that are mediated by the protein C (PC) cellular pathway (Mosnier et al., 2007). The anticoagulant action of APC is mediated by the irreversible proteolytic inactivation of the coagulant factors Va and VIIIa with contributions from various cofactors. Independent of its anticoagulant activity, APC exerts direct cytoprotective effects resulting in: (i) cytoprotective alteration of gene expression profiles; (ii) anti-inflammatory activities; (iii) anti-apoptotic activity; and (iv) protection of endothelial barriers (Joyce et al., 2001; Finigan et al., 2005). Recent studies suggest that APC protects against diabetic endothelial and glomerular injury (Isermann et al., 2007), multiple sclerosis (Han et al., 2008), ischemia reperfusion injury in the kidney and lung (Mosnier et al., 2007), spinal cord ischemia (Yamauchi et al., 2006), and retinal ischemia (Du et al., 2011). Other mechanisms have been proposed to explain the efficacy of APC, including intracellular survival signals such as phosphorylated serine-threonine kinase (p-Akt) (Yamauchi et al., 2006). Therefore, we hypothesized that APC is a promising therapeutic approach for the treatment of NIHL. In this study, we assessed the protective effects of APC and investigated the possible mechanisms, including apoptosis markers and p-Akt, after NIHL.

2. Results

2.1. Auditory brainstem response

The ABR thresholds before noise exposure were essentially equivalent in all ears. No significant differences were observed in the baseline auditory thresholds among all groups at any frequency (Fig. 1). The time course change for the threshold shift after noise exposure is shown in Fig. 1. Immediately after exposure to noise (Day 0), the initial threshold shifts were elevated to the same level in all groups. No significant difference was found in the threshold shift between groups, and no protective effects could be detected by ABR analysis at Day 0. At 7 days after noise exposure (Day 7), significant differences were observed between Group 1 (non-treated) and Group 3 (APC: 300 U/kg) at 8, 12, 16, 20, and 32 kHz frequencies ($P<0.05$, Mann–Whitney U-test). At 14 days after noise exposure (Day 14), a significant difference was found between Group 1 (non-treated) and Group 3 (APC: 300 U/kg) at 8, 12, 16, 20, and 32 kHz frequencies ($P<0.05$, Mann–Whitney U-test). The threshold shift in Group 3 (APC: 300 U/kg) was lower compared to the shifts in Group 1 (non-

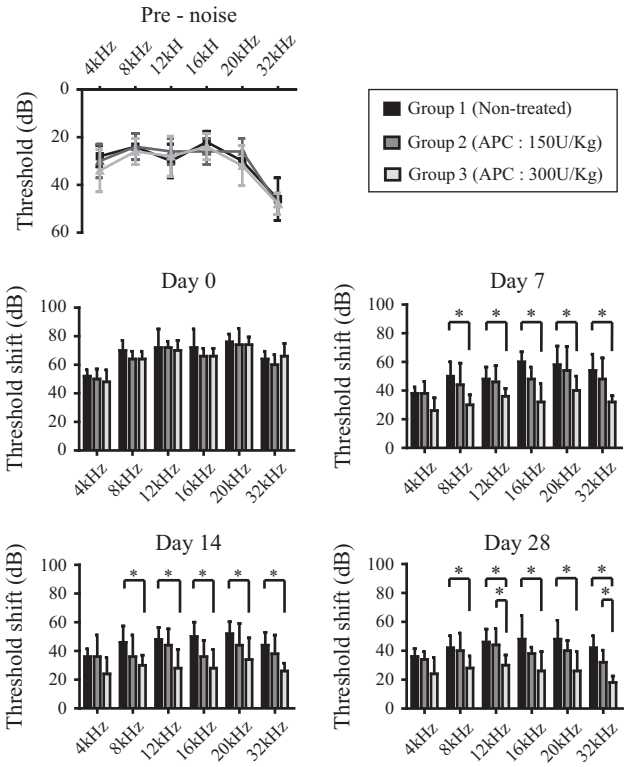

Fig. 1 – Auditory brainstem response (ABR) thresholds (mean with SE) measured immediately after noise exposure (Day 0) and at 7, 14, and 28 days after exposure in each group. Non-treated animals (Group 1) showed larger ABR threshold shifts compared with the APC-treated animals (Group 2 and 3). This indicates that APC provides a significant reduction in the ABR threshold shifts after noise exposure. (*$P<0.05$, nonparametric Dunnett's test).

treated) and Group 2 (APC: 150 U/kg) at 28 days after noise exposure (Day 28). Furthermore, significant differences were observed between Group 1 (non-treated) and Group 3 (APC: 300 U/kg) at 8, 12, 16, 20, and 32 kHz frequencies, and between Group 2 (APC: 150 U/kg) and Group 3 (APC: 300 U/kg), at 12 and 32 kHz frequencies ($P<0.05$, Mann–Whitney U-test). In this study, we used an octave-band noise centered at 4 kHz at a sound pressure level (SPL) of 126 dB for 5 h to induce acoustic injury, and the frequency-threshold shift curve of the ABR threshold shift showed a flat pattern because the noise exposure intensity was very high (Tabuchi et al., 2010).

2.2. Distortion product otoacoustic emissions

Distortion product otoacoustic emission (DPOAE) levels in response to 2 primary tones ($L1$, $L2=65$, 55 dB SPL and $f2/f1=1.22$) in the frequency range of 8–16 kHz were recorded in all groups. DPOAE originate from nonlinear mechanical sound processing in the inner ear, mainly due to normal outer hair cell function. Outer hair cell impairment can be detected by means of DPOAE. The frequency distribution of the DPOAE levels before noise exposure, immediately after noise exposure, and at 7, 14, and 28 days after exposure in ears with a detectable DPOAE response is shown in Fig. 2. Initially, the DPOAE levels were not significantly different