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Geranylgeranylacetone suppresses noise-induced expression of proinflammatory cytokines in the cochlea

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ABSTRACT

Objective: Heat shock transcription factor 1 (HSF1) is a master regulator of heat shock response, and also inhibits expression of inflammatory cytokines directly or indirectly. Here, we examined effects of HSF1 activation on the expression of proinflammatory cytokines in mouse cochlea after exposure to noise.
Methods: Male CBA/N mice with normal Preyer's reflex were exposed to intense noise for 3 h. Three hours after noise exposure, bilateral cochleae were removed and expression of major inflammatory cytokines was examined.
Results: We found that interleukin-6 (IL-6) and interleukin-1β (IL-1β) expression increased significantly after noise exposure, and the expression was suppressed significantly in mice administered with geranylgeranylacetone (GGA), which activates HSF1. Seven days after noise exposure, thresholds for auditory brainstem response were elevated, and GGA administration significantly suppressed this elevation.
Conclusion: These results suggest that HSF1-mediated suppression of proinflammatory cytokines in the cochlea by GGA administration could be an important means of inner ear protection.

1. Introduction

Heat shock proteins (Hsps) act as molecular chaperones to assist protein folding and suppress protein denaturation [1,2]. Hsps are induced in response to denaturation of cellular proteins, which may be caused not only by exposure to high temperature but also by a variety of other stresses including tissue ischemia [3] and anticancer drug treatment [4]. The mammalian heat shock response is regulated mainly at the level of transcription by heat shock transcription factor 1 (HSF1), which induces the expression of major Hsps and plays a major role in protecting cells from damage [5]. In the inner ear, sound exposure also induces a heat shock response with induction of Hsp70 in the cochlea [6] and HSF1 is required for sensory hair cell survival after acoustic overexposure [7,8], probably by inducing expression of major Hsps [9,10].

The antiulcer drug geranylgeranylacetone (GGA) has been known to activate HSF1 and induce Hsp expression [11,12]. Administration of GGA has a protective effect that is associated with Hsp upregulation in various organs such as the gastric mucosa, small intestine, spiral nerve, liver, heart, brain and retina [12–17]. About inner ear, we showed previously that GGA administration could prevent hair cell loss and hearing impairment due to noise exposure in the guinea pig and attenuate progressive hearing loss in a model of age-related hearing loss via induction of major Hsps [18,19].

Noise exposure induces expression of major inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) which may mediate noise injury in rat cochlea [20]. It was also reported that IL6-receptor emerged in cochlea (hair cell, supporting cell, spiral ganglion) when mouse was exposed to intense sound [21] and anti-IL6 receptor antibody (MR16-1) could attenuate noise injury. These results indicate that regulation of IL-6 leads to protective effect on cochlea protection.

Interestingly, HSF1 is involved in inflammatory responses by directly or indirectly regulating these three proinflammatory cytokine genes [22–25]. These facts give us hypothesis that HSF1 may suppress noise injury by inhibiting inflammatory cytokine expression. Here we show that administration of GGA suppresses the expression of IL-6 and IL-1β after noise injury, and inhibits hearing loss.

Abbreviations: ABR, auditory brainstem response; GGA, geranylgeranylacetone; HSF1, heat shock transcription factor 1; Hsp, heat shock protein; IL-6, interleukin-6; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor α.

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2. Materials and methods

2.1. Animal preparation

32 mice were used in this study. Male CBA/N mice (25–30 g body weight) were housed in cages in a room maintained at 24 °C on 12 h light/dark cycle without noise. Mice used in the study had normal Preyer's reflexes and a normal tympanic membrane.

GGA was administered with the chow to 8 weeks old animals for 2 months. GGA granules (Selbex®, Eisai Co., Ltd., Tokyo, Japan) were mixed with powdered rodent chow at a concentration of 0.5%, corresponding to an intake of 400–600 mg/kg/day during the study [26]. GGA induces Hsps in the mice cochlea on these treatment conditions [19]. This study was reviewed by the Institutional Animal Care and Use Committee (IACUC) at Yamaguchi University, and was carried out under the control of the Rule for the Care and Use of Laboratory Animals at Yamaguchi University and of Law No. 105, Notification No. 88 and Guideline No. 71 of the Japanese Government.

2.2. Noise exposure

Mice were exposed to intense octave band noise (130 dB sound pressure level) with a center frequency of 4 kHz for 3 h under pentobarbital anesthesia (3 mg/kg i.p.). Each animal was fixed in a chamber with a speaker (JBL professional series, model No. 2446H) centered above its head at a distance of 15 cm. Sound intensity was monitored near the external auditory canal with a sound level meter (NA-60, Rion, Tokyo, Japan).

2.3. Sample collection

3 h after noise exposure, the animals were decapitated under deep anesthesia with an overdose of pentobarbital. Using scissors, the cochleae were immediately dissected at the hook region and frozen in liquid nitrogen. Within a few minutes the tissue was placed in 1 ml of Trizol® reagent (Invitrogen, Carlsbad, CA) for RNA extraction according to the manufacturer's protocol.

2.4. Reverse transcriptase-PCR and realtime PCR analyses

cDNA was synthesized using cloned AMV First Strand Synthesis Kit (Invitrogen, Carlsbad, CA), then PCR-amplified using the primer sets. Conventional PCR was performed using Ex Taq polymerase (Takara, Japan). Amplified DNA fragments were resolved by agarose gel electrophoresis, stained with ethidium bromide and photographed using Epi-Light UV FA1100 (Aisin Cosmos, Japan). Levels of mRNA were estimated by densitometry of the bands using the public domain image processing program Image J (http://rsbweb.nih.gov/ij/download.html). For quantitative realtime RT-PCR analysis, cDNA (1 μg) was amplified using the QuantiTect SYBR Green PCR Kit (QIAGEN, Germany) in a LightCycler instrument (Roche Applied Science, Mannheim, Germany). Crossing point values were calculated by the second derivative maximum method using LightCycler Software v3.5. Realtime PCR data were normalized to GAPDH, and are presented as mean ± standard error of the mean.

2.5. Auditory brainstem responses

The ABR thresholds of all mice were assessed under pentobarbital anesthesia (3 mg/kg i.p.) 7 days after noise exposure. Subcutaneous stainless steel electrodes were placed at the vertex (positive) and antiunion (negative); the lower back served as ground. The sound stimuli consisted of 32, 16, 8, 4 and 2 kHz tone bursts (rise-fall time 2 ms, duration 4 ms) which were presented to the external auditory canal via a 10 cm tube connected to the earphone. The stimulus intensity was evaluated with a sound level meter (NA-60) adjacent to the tip of this tube. Responses to stimuli were recorded using a PowerLab data acquisition system (ADInstruments, NSW, Australia). ABR thresholds were defined as the lowest stimulus intensity producing a reliable peak 3 in ABR waveforms.

2.6. Statistical analysis

Data are expressed as mean ± SEM. The difference in the ABR thresholds and expression of cytokines were analyzed GraphPad Prism version 4.0c software for Macintosh (GraphPad, USA). The difference in these values among the groups were assayed, using Mann–Whitney test. P values less than 0.05 were accepted as statistically significant.

3. Results

3.1. Induction of proinflammatory cytokine expression after noise exposure, and its suppression by GGA administration

Animals were divided into four groups (Table 1): control group A which was fed a normal rodent chow, group B which was fed normal chow and also subjected to noise exposure for 3 h, group C which was fed chow containing GGA for 2 months, and group D which was given the GGA-containing chow for 2 months and was also noise-exposed for 3 h.

Fig. 1 shows that the band of Hsp70 mRNA expression was slightly detected in the control group A and strongly induced after noise exposure (group B), which was considered heat shock response. Following GGA administration, Hsp70 expression was moderately increased without intense sound stress (group C), which demonstrating that GGA alone could induce the heat shock response in the mouse cochlea. Drastically, Hsp70 expression in GGA-treated animals was heightened even further by noise exposure (group D). We examined cytokine gene expression in the experimental groups (Fig. 1). IL-6 mRNA was induced by noise exposure, but this response was diminished in the GGA-treated animals. IL-1β was induced both by noise exposure and by GGA treatment, whereas it was repressed following noise exposure in the GGA-treated animals. Relative mRNA quantities estimated from these data by densitometry are shown in Fig. 1b. In contrast to these two inflammatory cytokines, the changes in TNF-α expression after noise exposure were not significant.

We confirmed these changes in expression levels of IL-6 and IL-1β using quantitative realtime-PCR analysis (Fig. 1c). In keeping with the results, IL-6 and IL-1β expression were significantly increased by noise exposure ($P < 0.05$) in mice given normal chow, the upregulation of IL-6 and IL-1β by noise exposure was suppressed by GGA administration.

3.2. Elevation of the auditory brainstem response threshold after noise exposure and its suppression by GGA administration

The auditory brainstem responses (ABR) of mice in the four groups were assessed 7 days after noise exposure (Fig. 2). Prior to

Table 1
Experimental groups.

	A	B	C	D
Noise exposure	–	+	–	+
GGA	–	–	+	+

Animals were divided into four groups as shown. Groups A and B were given only powdered rodent chow. Groups C and D were given powdered rodent chow containing 0.5% GGA for 2 months. Groups B and D were subjected to noise exposure for 3 h as described in Section 2.2.