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Ceramide-1-phosphate protection of cochlear hair cells against cisplatin ototoxicity

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ABSTRACT

Background: Ceramide-1-phosphate (C1P) is a phosphorylated form of ceramide. While ceramide is known to be an inducer of apoptosis of cochlear hair cells in cisplatin ototoxicity, little is known about the function of C1P in cochlear diseases.
Purpose: The present study was designed to examine whether C1P could protect cochlear hair cells against cisplatin ototoxicity.
Materials and methods: Explants of cochlear basal turns collected from C57BL/6J mice at postnatal days 3–5 were used in all experiments. Cochlear explants were exposed to 5 or 10 μM cisplatin for 48 h to assess the effects of C1P, NVP-231 (a ceramide kinase inhibitor), or ceramide. Western blotting of pAkt/Akt and pMAPK/MAPK was examined to check whether this pathway was modulated by C1P.
Results: C1P activated the Akt and MAPK pathway and significantly reduced cochlear cell death induced by cisplatin. Coadministration of cisplatin and ceramide significantly increased cochlear hair cell death. In addition, when treating cochlear hair cells with NVP-231 in the presence of cisplatin or ceramide, a remarkable increase in apoptosis of hair cells was observed.
Conclusion: The present findings confirmed the protective effects of C1P in the cisplatin ototoxicity. The balance between ceramide and C1P may play a critical role in the determination of hair cell fate in cisplatin ototoxicity.

1. Introduction

Cochlear hair cells have a crucial function to convert sound signals to electric signals in the auditory system; however, they are very sensitive to harmful stimulations such as cisplatin used in chemotherapy.

Ceramide is generally formed either via de novo synthesis from serine and palmitoyl-CoA or via hydrolysis of sphingomyeline by sphingomyelinase [1,17]. It was demonstrated that cisplatin activates sphingomyelinase, which triggers the release of ceramide [18]. Although ceramide is known to be an inducer of apoptosis in aminoglycoside ototoxicity [19], the effects of ceramides on cisplatin ototoxicity have never been examined.

Ceramide-1-phosphate (C1P) is synthesized by phosphorylation of ceramide in mammalian cells [6]. Currently, ceramide kinase (CERK) is the only known mammalian enzyme to have this function. Since the first biological activity of C1P relating to DNA synthesis and cell division was identified [10,11], researchers have found that this phospholipid was attributed to various vital functions, such as macrophage proliferation and migration [7,14], phagocytosis [15], inflammation [22,23] and cell protection [12,13]. However, it has not been revealed yet whether C1P could inhibit cisplatin-induced cochlear hair cell death.

Induction of cell death is a complex process and tightly regulated. In the present study we hypothesized that C1P could be an antiapoptotic molecule and that targeted ceramide/C1P balance could interfere cochlear cell survival.

2. Methods

2.1. Culture technique

2.1.1. Cochlear explants

The lower basal turn of the organ of Corti was dissected from C57BL/6J mouse on postnatal days 3 (P3) to 5 (P5) and cultured

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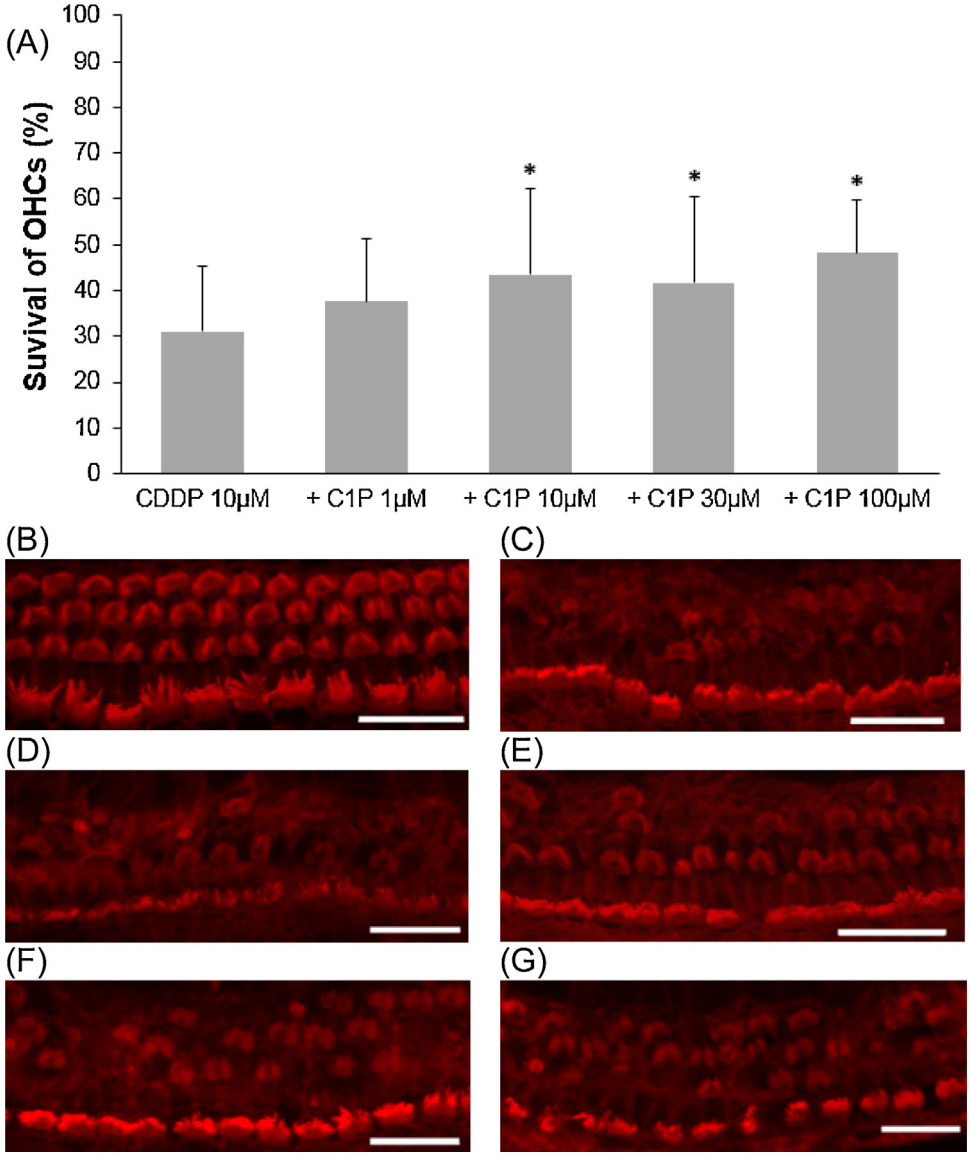

Fig. 1. Effects of C1P on the survival of cochlear outer hair cell in cisplatin ototoxicity. (A) Quantitative analysis of cochlear outer hair cells. The survival of cochlear outer hair cells significantly increased at 10 μM C1P or higher (ANOVA followed by Bonferroni post hoc test, $^{*}P < 0.05$). (B – G) Representative photographs of each group (scale bar 20 μm). (B) 100 μM C1P alone. (C) 10 μM cisplatin alone. (D) 10 μM cisplatin and 1 μM C1P. (E) 10 μM cisplatin and 10 μM C1P. (F) 10 μM cisplatin and 30 μM C1P. (G) 10 μM cisplatin and 100 μM C1P. CDDP: cisplatin.

according to the methods of Van de Water and Ruben [29] and Sobkowicz et al. [25]. All animal procedures were carried out according to the guidelines of the Laboratory Animal Research Center of Tsukuba University.

2.1.2. Cisplatin treatment

Cochlear explants were maintained in a culture medium containing Dul-becco's modified Eagle's medium (DMEM), 10% fetal bovine serum (FBS), 25 mM HEPES, and 30 U/mL penicillin. They were cultured in an incubator at 37 °C with 5% CO_2 at 95% humidity. Cochlear explants were maintained in the culture medium overnight (8–12 h) and then were exposed to the culture medium containing cisplatin (Nichi-Iko, Sogawa, Japan) for 48 h [27].

Cisplatin-induced ototoxicity was characterized by the missing of cochlear outer hair cells at the basal turn of the organ of Corti. Before conducting this study, we examined the damage of cochlear outer hair cells by exposing cochlear explants to several concentrations of cisplatin from 1 to 50 μM. The concentrations of cisplatin at 5 and 10 μM were chosen for the present experiments.

2.1.3. C1P treatment

C1P (Sigma, St. Louis, MO, USA) was initially dissolved in ethanol at a concentration of 2 mg/ml, and then diluted in the culture medium to the final concentrations before use. After the cochlear explants were stabilized in the culture medium overnight, each group (from 9 to 18 cochlear explants) was exposed to culture media containing 10 μM cisplatin plus various concentrations of C1P (from 1 to 100 μM) for 48 h.

2.1.4. Ceramide treatment

C16-ceramide (LKT Laboratories, St. Paul, MN, USA) was dissolved in ethanol at a concentration of 5 mg/ml. The effect of ceramide alone on cochlear explants was tested using concentrations from 10 to 500 μM. Later, the combination of 5 μM cisplatin and various concentrations of ceramide (from 10 to 500 μM) was