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Anti-apoptotic effect of phloretin on cisplatin-induced apoptosis in HEI-OC1 auditory cells

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Abstract:
Cisplatin is a highly effective chemotherapeutic agent, but it has significant ototoxic side effects. Apoptosis is an important mechanism of cochlear hair cell loss following exposure to cisplatin. The present study examined the effects of phloretin, a natural polyphenolic compound found in apples and pears, on cisplatin-induced apoptosis. We found that phloretin induced the expression of heme oxygenase-1 (HO-1) protein in a concentration- and time-dependent manner. Phloretin induced nuclear factor-E2-related factor 2 (Nrf2) nuclear translocation, and dominant-negative Nrf2 attenuated phloretin-induced expression of HO-1. Phloretin activated the JNK, ERK and p38 mitogen-activated protein kinase (MAPK) pathways, and the JNK pathway played an important role in phloretin-induced HO-1 expression. Phloretin protected the cells against cisplatin-induced apoptosis. The protective effect of phloretin was abrogated by zinc protoporphyrin IX (ZnPP IX), a HO inhibitor. Furthermore, phloretin pretreatment inhibited mitochondrial dysfunction and the activation of caspases. These results demonstrate that the expression of HO-1 induced by phloretin is mediated by both the JNK pathway and Nrf2; the expression inhibits cisplatin-induced apoptosis in HEI-OC1 cells.

Key words:
phloretin, cisplatin, heme oxygenase-1, JNK, caspase

Abbreviations: DMEM – Dulbecco's modified Eagle's medium, FBS – fetal bovine serum, HEI-OC1 – House Ear Institute-Organ of Corti 1, HO-1 – heme oxygenase-1, MAPKs – mitogen-activated protein kinases Nrf2 – nuclear factor-E2-related factor 2, PKC – protein kinase C, ZnPP – zinc protoporphyrin

Introduction

Cisplatin (*cis*-diamminedichloroplatinum II, CDDP) is a highly effective chemotherapeutic agent used to treat several types of solid tumors [15]. However, reversible and irreversible side effects, including ototoxicity and nephrotoxicity, can limit its utility and therapeutic profile [3, 20]. Therefore, many researchers have tried to ameliorate the ototoxic side effect of cisplatin. The loss of hearing appears to result from the destruction of outer hair cells in the organ of Corti as a result of apoptosis [12]. Several studies have indicated that antioxidants are effective in the prevention of drug-induced hearing loss [4, 33]. Indeed, antioxidants have shown efficacy in the attenuation of noise-induced hearing loss, protecting auditory outer hair cells and electrophysiological responsiveness [33].

Heme oxygenase (HO)-1 is a critical factor in response to oxidative injury, a major result of which is the degradation of heme to biliverdin, iron, and car-

bon monoxide [24, 36]. Previous studies have demonstrated that HO-1 works as a part of the cytoprotective mechanism, which includes antioxidant [10], anti-inflammatory [17, 23], antiproliferative [11], and antiapoptotic properties [8]. Thus, considering the cytoprotective role of HO-1, the induction of HO-1 expression by pharmacological modulation may represent a novel target for therapeutic treatments of various diseases.

Phloretin, a natural polyphenolic compound found in apples and pears, has been shown to exert antitumor activity through inhibition of protein kinase C (PKC) activity and induction of apoptosis [25]. Phloretin has been reported to be a hepatoprotective agent, with studies showing that it prevents tacrine-induced cytotoxicity in human liver cancer cells [2]. Phloretin has potent antioxidant activity and antiproliferative effects on cancer cells [13, 31]. However, little is known about the protective mechanism by which phloretin rescues cells from oxidative stresses. Recently, attention has been focused on the effect of HO-1 induced by phytochemicals investigating the role of HO-1 against oxidative cell damage [30]. In this study, our aim was to elucidate the molecular mechanism of phloretin protection against cisplatin-induced apoptosis in HEI-OC1 cells, specifically focusing on the upregulation of HO-1.

Materials and Methods

Materials

Phloretin and cisplatin were from Sigma-Aldrich (St. Louis, MO, USA). Zinc protoporphyrin IX (ZnPP IX), an inhibitor of heme oxygenase activity, was purchased from Porphyrin Products (Logan, UT, USA). PD098059, SB203580, SP600125, and Anti-HO-1 antibody were purchased from Calbiochem (San Diego, CA, USA). Antibodies against Bcl-2, Bax, and Nrf2 were purchased from Santa Cruz Biotechonology (Santa Cruz, CA, USA). Anti- phospho-JNK, -ERK, and -p38 antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Unless indicated otherwise, all other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Cell culture

The establishment and characterization of the conditionally immortalized House Ear Institute-Organ of Corti 1 (HEI-OC1) cells were described by Kalinec et al. [22]. HEI-OC1 cells have recently been established from long-term cultures of Immorto-mouse cochlea and characterized. Cells were maintained in high-glucose Dulbecco's modified Eagle's medium (DMEM; GIBCO BRL, NY, USA) supplemented with 10% fetal bovine serum (FBS; GIBCO BRL, NY, USA) at 33°C in a humidified incubator with 5% CO_2.

Cell viability

Cells were subcultured in 96-well plates at a density of 5×10^4 cells/well. Cells were treated with cisplatin in the presence or absence of phloretin or ZnPP. The MTS assay was performed with the CellTiter 96 Aqueous nonradioactive cell proliferation assay kit (Promega Corp., WI, USA), according to the manufacturer's instructions. The absorbance was read at 490 nm on an ELISA reader, and the percentage of cell survival was determined.

Preparation of cytosolic and nuclear extracts

Cytosolic and nuclear fractions were prepared as previously described [32]. Briefly, cells were washed three times with cold PBS and centrifuged at 130 × g for 10 min. The pellet was carefully resuspended in 3 pellet volumes of cold buffer containing 20 mM HEPES, pH 7.0, 0.15 mM EGTA, 10 mM KCl, 1% Nonidet-40, 1 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, 20 mM NaF, 1 mM sodium pyrophosphate, and 1 mM Na_3VO_4. The homogenate was then centrifuged at 500 × g for 20 min, and the nuclear pellet was washed in 5 pellet volumes of cold PBS. After centrifugation at 500 × g for 20 min, nuclei were resuspended in 2 pellet volumes of cold hypertonic buffer containing 10 mM HEPES, pH 8.0, 25% glycerol, 0.4 M NaCl, 0.1 mM EDTA, 1 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, 20 mM NaF, 1 mM sodium pyrophosphate, and 1 mM Na_3VO_4 and incubated for 30 min at 4°C on a rotating wheel. Nuclear debris was removed by centrifugation at 900 × g for 20 min at 4°C. The supernatant was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and analyzed by Western blot analysis with anti-Nrf2 and anti-Lamin B antibodies.