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Review article

Mechanisms of cisplatin-induced ototoxicity and prevention

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Abstract

Cisplatin is a widely used chemotherapeutic agent to treat malignant disease. Unfortunately, ototoxicity occurs in a large percentage of patients treated with higher dose regimens. In animal studies and in human temporal bone investigations, several areas of the cochlea are damaged, including outer hair cells in the basal turn, spiral ganglion cells and the stria vascularis, resulting in hearing impairment. The mechanisms appear to involve the production of reactive oxygen species (ROS), which can trigger cell death. Approaches to chemoprevention include the administration of antioxidants to protect against ROS at an early stage in the ototoxic pathways and the application of agents that act further downstream in the cell death cascade to prevent apoptosis and hearing loss. This review summarizes recent data that shed new light on the mechanisms of cisplatin ototoxicity and its prevention.

Keywords: Cisplatin; Reactive oxygen species; Apoptosis; NADPH; Oxidase

1. Introduction

Cisplatin is a highly effective chemotherapeutic agent that is widely used to treat a variety of soft tissue neoplasms, including ovarian, testicular, cervical, head and neck, lung and bladder cancer. Serious side effects include nephrotoxicity, neurotoxicity and ototoxicity. In order to effect cures, the dosing of cisplatin has been increased in recent treatment protocols. Some audiometric studies have reported elevated hearing thresholds in 75–100% of patients treated with cisplatin (McKeage, 1995). This is particularly problematic in children receiving cisplatin. Risk factors that increase the risk for ototoxicity from cisplatin in children include: younger age, larger cumulative doses, pre-existing hearing loss and renal disease (Li et al., 2004; Knight et al., 2005) and irradiation of the brain or skull base (Chen et al., 2006).

Ototoxicity of cisplatin can be reduced by various protective agents. This paper reviews recent research findings that provide new insights into the mechanisms for cisplatin ototoxicity and the effects of various protective agents that may ameliorate cisplatin ototoxicity.

2. Effects on cochlear function

Adverse effects of cisplatin on auditory function have been documented in numerous reports. Ototoxicity has been demonstrated in animal experiments by reduction of the endocochlear potential (EP) (Ravi et al., 1995; Tsukasaki et al., 2000; Klis et al., 2000) and elevation of the thresholds for both the compound action potential (CAP)

Abbreviations: AAV, adeno-associated virus; ABR, auditory brainstem response; AUC, area under the curve; BK, big conductance potassium; EP, endocochlear potential; CAP, compound action potential; CM, cochlear microphonic; GFP, green fluorescent protein; HMG1, high mobility group; iNOS, inducible nitric oxide synthase; KIM-1, kidney injury molecule; NOX-3, isoform of NADPH oxidase; ROS, reactive oxygen species; TUNEL, terminal nucleotidyl transferase-mediated dUTP-biotin nicke end-labelling; XIAP, the X-linked inhibitor of apoptosis protein

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and cochlear microphonic (CM) after ototoxic doses of cisplatin. The CAP amplitude is reduced to a greater extent than the (CM) amplitude. The greater effect of cisplatin on the CAP threshold may result from several changes induced within the cochlea. These include detachment of the myelin sheath of spiral ganglion cells, alteration of hair cell function and reduction of the EP. This would explain why cisplatin-treated guinea pigs can exhibit a large discrepancy between the elevation of CAP threshold in the presence of low CM thresholds. Thus, the CAP threshold shift cannot be explained completely by the changes in CM threshold (Van Ruijven et al., 2005a). Distortion product otoacoustic emissions are diminished in cisplatin-treated gerbils (Alam et al., 2000). Auditory brainstem responses in cisplatin-treated animals demonstrate increased thresholds, with greatest effects in the higher frequencies (Rybak et al., 2000).

3. Effects on cochlear morphology

Cisplatin ototoxicity has been shown to have at least three major tissue targets in the cochlea: organ of Corti, spiral ganglion cells and lateral wall (stria vascularis and spiral ligament). Studies in guinea pig reveal that cisplatin affects both the organ of Corti (primarily the outer hair cells) and the spiral ganglion cells (Van Ruijven et al., 2005a). Type I spiral ganglion cells undergo detachment of their myelin sheaths. The time sequence of damage to spiral ganglion cells and outer hair cells in guinea pig follow a similar time course, suggesting that injury to both cochlear areas occurred in parallel, rather than sequentially (Van Ruijven et al., 2005a).

Cisplatin ototoxicity in rats was manifested by deleterious effects on the basal turn stria vascularis, including strial edema, bulging, rupture and compression of the marginal cells and depletion of organelles from the cytoplasm (Meech et al., 1998). Guinea pigs allowed to recover for more than four weeks after cisplatin administration demonstrated shrinkage in the area of the stria, even though the EP had recovered by this time. This shrinkage was caused by a decrease in the intermediate cell area and, to a lesser extent, by a decrease in the marginal cell area (Sluyter et al., 2003).

TUNEL staining was used to detect DNA damage (breaks in double-stranded DNA) in the cochlea of cisplatin-treated gerbils. TUNEL is an acronym for terminal nucleotidyl transferase-mediated dUTP-biotin nick end-labelling and represents the *in situ* end-labelling or transfer of biotinylated nucleotide to the 3''-OH end of DNA. It is frequently used as a marker for apoptotic cell death. Positive TUNEL staining indicates that the cell nucleus is undergoing degeneration and likely undergoing apoptosis. Additional staining with Hoechst 33432 is used to identify cells undergoing apoptosis. Cell stained with the latter that demonstrate pyknotic and condensed nuclei are classified as apoptotic (Alam et al., 2000). Apoptosis of cells in the organ of Corti, primarily the outer hair cells, and spiral ganglion cells in the basal turn of the gerbil cochlea occurred after cisplatin administration. On the other hand, the stria vascularis demonstrated TUNEL-positive staining in all three turns (Alam et al., 2000). The ototoxic effect of cisplatin on lateral wall tissues in the gerbil was further substantiated with *in vitro* studies of the spiral ligament. Gerbil type I spiral ligament cells also undergo significant apoptosis after cisplatin exposure in cell culture. This was caused by cisplatin blocking BK channels (Liang et al., 2005). Administration of a marginally ototoxic dose of cisplatin (10 mg/kg) resulted in positive TUNEL staining in the stria vascularis of guinea pigs; however, no TUNEL staining was detected in hair cells. This dose of cisplatin was probably not sufficient to cause hair cell death (Watanabe et al., 2003).

Platinated DNA was immunolocalized to the nuclei of outer hair cells, supporting cells of the organ of Corti, marginal cells of the stria vascularis and the cells in the spiral ligament of the basal turn. This was demonstrated using a polyclonal rabbit serum containing antibodies that recognize cisplatin–DNA adducts. These antibodies also stained samples of renal cortex, which served as positive controls (Van Ruijven et al., 2005b). This finding confirms that these cells are targets for cisplatin toxicity. However, a more recent study by Thomas et al. (2006) demonstrated immunostaining for platinum–DNA adducts primarily in the marginal cells of the stria vascularis. In contrast to the study by Van Ruijven et al. (2005b), the investigation by Thomas et al. (2006) showed no specific accumulation of cisplatin–DNA adducts. The discrepancies between the findings in these two studies in the guinea pig could be attributed to the dosing protocol used, the antibodies used to detect platinated DNA in the tissues and the quality of tissue preservation after cisplatin. Van Ruijven et al. (2005b) employed albino Dunkin–Hartley guinea pigs and administered cisplatin at a dose of 2 mg/kg by intraperitoneal (i.p.) injection five times per week for a period of two weeks, resulting in a cumulative dose of 20 mg/kg, and sacrificed the animals 48 h after the last dose. Thomas et al. (2006) treated pigmented guinea pigs of the same strain with 12.5 mg/kg i.p. and sacrificed the animals at 8, 24 or 48 h after cisplatin injection. A polyclonal antibody was used by Van Ruijven et al. (2005b) as opposed to a monoclonal antibody directed against platinum–guanine–guanine intrastrand cross-links. The polyclonal antiserum also contained antibodies that react with other nuclear antigens besides cisplatin–DNA adducts. The polyclonal antiserum was preabsorbed with a homogenate of non-treated guinea pig kidneys prior to immunostaining the cochlea (Van Ruijven et al., 2005b). Because of mild fixation and extensive proteolytic digestion, the hair cells were less well preserved in the study by Thomas et al. (2006) preventing a systematic evaluation of these cells. These studies should be repeated using monoclonal antibodies with better tissue preservation of the organ of Corti.