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Cisplatin ototoxicity to the rat inner ear: A role for HMG1 and iNOS

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Abstract

Cisplatin is a chemotherapeutic agent that causes toxic damage to the inner ear (ototoxicity). Although much attention has been directed at identifying ways to protect the inner ear against cisplatin ototoxicity, little is known about the mechanisms by which cisplatin causes damage to the inner ear. Binding of high-mobility group (HMG1) protein to cisplatin-modified DNA participates in mediating the antitumor effects of cisplatin (Ohndorf U-M, Rould MA, He Q, Pabo CO, Lippard SJ. Basis for recognition of cisplatin modified DNA by high-mobility-group proteins. Nature 1999;399:708–12). This study seeks to determine if HMG1 may also participate in the ototoxicity of cisplatin. To address this, patterns and levels of expression of HMG1 have been evaluated in the rat cochlea in response to cisplatin chemotherapy. Our findings demonstrate a marked upregulation of HMG1 protein in the spiral (auditory) ganglion cells of cisplatin-treated rats in comparison to levels of expression of HMG1 in the spiral ganglion cells of untreated control animals. Increased levels of HMG1 were observed in the cisplatin-treated kidney, a peripheral target tissue of cisplatin, but not in the heart, a tissue not typically affected by cisplatin chemotherapy, suggesting HMG1 specificity in cisplatin toxicity. Furthermore, levels of inducible nitric oxide synthase (iNOS), an HMG-regulated enzyme associated with cochlear pathology, are increased in the spiral ganglion cells of cisplatin-treated rats 1 day post the cisplatin-mediated upregulation in HMG1. This increase in HMG1 and iNOS can be prevented in the cochleae of cisplatin-treated rats by administration of L-methionine, an established method of protection against cisplatin ototoxicity (Li G, Frenz DA, Brahmblatt S, Feghali JG, Ruben RJ, Berggren D, et al. Round window membrane delivery of L-methionine provides protection from cisplatin ototoxicity without compromising chemotherapeutic efficacy. NeuroToxicology 2001;22:163–76). Our results support a role for HMG1 and iNOS in mechanisms of cisplatin ototoxicity in the rat inner ear.

Keywords: Cisplatin toxicity; Inner ear; HMG1; iNOS; L-Methionine

1. Introduction

Cisplatin is a chemotherapeutic agent effective in the treatment of both adult and pediatric malignancies, including breast, testicular, ovarian, head and neck, and uterine cervix carcinomas (Gandara et al., 1989; Smith and Talbot, 1992; Harrison et al., 1991). However, cisplatin has ototoxic properties. In patients receiving cisplatin chemotherapy, ototoxicity is characterized by a loss of auditory hair cells, degeneration of the stria vascularis, a significant decrease in spiral ganglion cells (Rybak et al., 1995), and an associated bilateral high frequency hearing loss (Ozols and Young, 1985; Anniko and Sobin, 1986). Similar ototoxic effects are observed in the stria vascularis and cochlea of cisplatin-treated animals (Estrem et al., 1981). Besides ototoxicity, nephrotoxicity is a limiting side effect of cisplatin (Safinstein et al., 1986).

Amelioration of cisplatin-induced ototoxicity has recently been achieved in animal models. Local inner ear administration of cytoprotective antioxidant agents such as D- or L-methionine (Ekborn et al., 2002; Li et al., 2001), trolox (Teranishi and Nakashima, 2003), and thiourea (Ekborn et al., 2003) can provide protection against the loss of outer auditory hair cells and/or loss of hearing acuity produced by cisplatin in the rat or guinea pig inner ear. Systemic administration of L-methionine (Li et al., 2001) or salicylate effectively attenuates cisplatin ototoxicity in the rat inner ear (Li et al., 2002), while systemic diethyldithiocarbamate (DDTC), 4-methylthiobenzoic acid (MTBA), and ebselen each protects against the depletion in glutathione, reduction in antioxidant enzyme activity, and

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elevation of the lipid peroxidation product malondialdehyde typically associated with cisplatin ototoxicity (Rybak et al., 2000). Neuroprotective effects of L-*N*-acetyl cysteine against cisplatin-induced auditory neuronal toxicity has been demonstrated in cochlear explants in culture (Feghali et al., 2001). In addition, gene therapy vector-mediated neurotrophin expression can attenuate cisplatin ototoxicity in vivo and in vitro, as measured by spiral ganglion cell survival (Bowers et al., 2002; Chen et al., 2001).

Central to the development of efficacious methods of protection against cisplatin ototoxicity is gaining an understanding of the mechanisms underlying the cytotoxic action of cisplatin. The mechanism of cisplatin action against target tumor cells is a consequence of the formation of covalent adducts between cisplatin and certain bases in DNA (Zlatanova et al., 1998). The biological activity of cisplatin cannot, however, be explained solely on the basis of its ability to damage DNA since the trans isomer of cisplatin binds DNA but is ineffectual as an antitumor agent (Pil and Lippard, 1992). Stereochemical differences in the DNA adducts formed by the two isomers suggest that the antitumor activity of cisplatin arises from interactions of the damaged DNA with cellular proteins that specifically recognize cisplatin-damaged DNA. These cellular proteins include members of the high-mobility group (HMG) protein family (Wunderlich and Bottger, 1997), including HMG1/2, HMG14/17, and HMGI. Besides functions which include binding to AT-rich DNA sequences, transcriptional regulation, and chromosomal replication (Thomas and Travers, 2001), HMG proteins are an important structural element of the cytoplasm (Mayer et al., 1997).

Elevated expression of HMGI has been demonstrated in cultured HeLa cells, transformed thyroid cells, and tumor cells in comparison to HMGI levels in normal or untransformed cells (Lund et al., 1983; Giancotti et al., 1989), suggesting an association between HMG and malignant phenotype. Consistent with this, HMGI is significantly increased in human neoplasms (Abe et al., 2000), while HMG1 and-2 are abundantly expressed in rapidly dividing and transformed cells (Wunderlich and Bottger, 1997). HMG1 recognizes structural distortion in DNA caused by cisplatin (Bruhn et al., 1992) and binds cisplatin-modified DNA. This binding of HMG1 to cisplatin-modified DNA is implicated in mediating antitumor properties of cisplatin (Ohndorf et al., 1999). A two-fold increase in HMG1 in human MCF-7 breast cancer cells has been correlated with a two-fold increase in the sensitivity of these cells to cisplatin (He et al., 2000a), implicating a role for HMG1 in conferring sensitivity of tumor cells to cisplatin (Jamieson and Lippard, 2000). It is therefore reasoned that HMG1 may play a potential role in mediating cisplatin sensitivity to peripheral target tissues, including the inner ear.

Cisplatin-induced damage to the rat cochlea is associated with the generation of reactive oxygen species (ROS) (Kopke et al., 1997). Nitric oxide is a free radical that is formed by the enzyme nitric oxide synthase (NOS), and that is associated both with cochlear physiology (Fessenden and Schact, 1998) and pathology (Nakagawa et al., 1999; Hess et al., 1999). The inducible isoform of NOS (iNOS) either is not found in the cochlea under normal conditions or is expressed at low levels, but is upregulated in damaged cochlear tissues in response to an appropriate toxic stimulus. Elevated levels of iNOS are associated with cisplatin-mediated cytotoxicity in the kidney, and can be attenuated by the anti-inflammatory cytokine interleukin-10 (Deng et al., 2001). Genes for iNOS are regulated by HMG proteins (Reeves and Beckerbauer, 2001).

Although oxidative stress-induced damage is implicated in mediating the ototoxicity of cisplatin, the precise mechanisms by which cisplatin causes damage to the inner ear have not yet fully been defined. This study investigates if the cytotoxic action of cisplatin against peripheral target tissues, like the antitumor activity of cisplatin, may be associated with altered expression of HMG1. Changes in HMG1 expression are demonstrated in the spiral ganglion and kidney in response to cisplatin chemotherapy, but not in tissue that is typically resistant to the cytotoxic effects of cisplatin (i.e. heart). Since expression of iNOS is controlled by HMG proteins (Reeves and Beckerbauer, 2001), patterns and levels of expression of iNOS are determined in the cochleae of cisplatin-treated and untreated control rats at time periods concurrent with and subsequent to the initial upregulation in HMG1 by cisplatin (i.e. days 2 and 3 post cisplatin treatment, respectively). The ability of systemic delivery of L-methionine, an established method of otoprotection against cisplatin ototoxicity (Li et al., 2001), to prevent alterations in HMG1 and iNOS levels is demonstrated. Our results suggest the participation of HMG1 and iNOS in mechanisms of cisplatin toxicity to the inner ear.

2. Materials and methods

2.1. Animals

A total of 20 seven-week-old Fisher 344 rats were used in this study, as follows: 8 rats (4 cisplatin-treated, 4 control) were used for the analysis of HMG1 and iNOS expression in the cochlea, kidney and heart; 12 rats (4 cisplatin-treated, 4 control, 4 cisplatin + L-methionine-treated) were used for the L-methionine otoprotection experiments. All rats were housed in our AAALAC-accredited animal facility, and all procedures were in accordance with the guidelines of NIH and the Albert Einstein College of Medicine.

2.2. Cisplatin treatment

Rats were anesthetized with a combination of ketamine (35 mg/kg, intramuscular [im]) and xylazine (15 mg/kg, im). Cisplatin diluted in 0.9% NaCl was injected intraperitoneally (ip) (Li et al., 2001) over 30 min in a single dose of 12 mg/kg. To improve hydration and lessen the nephrotoxic effects of cisplatin, rats received 4 ml of 0.9% NaCl solution at body temperature by subcutaneous injection twice a day from the day of cisplatin administration to day 3 post cisplatin administration.