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Review

Nicotinamide adenine dinucleotide: An essential factor in preserving hearing in cisplatin-induced ototoxicity

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ABSTRACT

Ototoxicity is an important issue in patients receiving cisplatin chemotherapy. Numerous studies have demonstrated that several mechanisms, including oxidative stress, DNA damage, and inflammatory responses, are closely associated with cisplatin-induced ototoxicity. Although much attention has been directed at identifying ways to protect the inner ear from cisplatin-induced damage, the precise underlying mechanisms have not yet been elucidated. The cofactor nicotinamide adenine dinucleotide (NAD^+) has emerged as an important regulator of cellular energy metabolism and homeostasis. NAD^+ acts as a cofactor for various enzymes including sirtuins (SIRTs) and poly(ADP-ribose) polymerases (PARPs), and therefore, maintaining adequate NAD^+ levels has therapeutic benefits because of its effect on NAD^+-dependent enzymes. Recent studies demonstrated that disturbance in intracellular NAD^+ levels is critically involved in cisplatin-induced cochlear damage associated with oxidative stress, DNA damage, and inflammatory responses. In this review, we describe the importance of NAD^+ in cisplatin-induced ototoxicity and discuss potential strategies for the prevention or treatment of cisplatin-induced ototoxicity with a particular focus on NAD^+-dependent cellular pathways.

1. Introduction

Cisplatin (*cis*-diamminedichloroplatinum (II)) was originally synthesized by Peyrone in 1845 and is often referred to as "Peyrone's chloride" (Rybak et al., 2005). In 1965, Rosenberg and Cavalieri discovered that platinum complexes were formed in the presence of ammonium and chloride ions and inhibited the proliferation of *Escherichia coli* (Rosenberg et al., 1965). In 1968, further tests using various bacteria showed cisplatin to be the most active tumor regressive compound in a tumor-bearing mouse model, and it was introduced into clinical trials for cancer therapy in 1971 (Lebwohl et al., 1998). Since the late 1970s, cisplatin has been widely used as a highly effective drug for the treatment of a variety of cancers including testicular, ovarian, cervical, bladder, and lung cancers but its clinical application was restricted by the occurrence of severe adverse effects such as nephrotoxicity, neurotoxicity, and ototoxicity (Bokemeyer et al., 1998; Pinzani et al., 1994). Cisplatin-induced nephrotoxicity can be partially attenuated by saline

Abbreviations: NAD^+, nicotinamide adenine dinucleotide; SIRT, sirtuin; PARP, poly(ADP-ribose) polymerase; OHCs, outer hair cells; ROS, reactive oxygen species; NOX, NAD(P)H oxidases; TNF, tumor necrosis factor; RANTES, regulated on activation normal T-cell expressed and secreted; MIP, macrophage inflammatory protein; MCP, monocyte chemoattractant protein; IL, interleukin; ICAM, intercellular adhesion molecule; 4-HNE, 4-hydroxynonenal; IDH2, isocitrate dehydrogenase 2; α-KGDH, α-ketoglutarate dehydrogenase; CBP, CREB binding protein; PCAF, P300/CBP-associated factor; Mdm2, mouse double minute 2; PUMA, p53-upregulated modulator of apoptosis; NOXA, NOX activator; PIG3, p53-induced gene 3; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IκB, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor; ERK, extracellular signal-regulated kinases; MAPK, mitogen-activated protein kinase; NQO1, NADH:-quinone oxidoreductase 1

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hydration or mannitol diuresis. However, there are no clinically proven protective remedies for cisplatin ototoxicity, which can be progressive and permanent. Although the incidence and severity of hearing loss after cisplatin treatment varies considerably, 40–80% of patients develop an elevated hearing threshold following cisplatin treatment (Bokemeyer et al., 1998; de Jongh et al., 2003; Knight et al., 2005). Therefore, it is imperative to develop treatments that will ameliorate cisplatin-ototoxicity.

Cisplatin ototoxicity is characterized by a loss of outer hair cells (OHCs) and spiral ganglion cells as well as degeneration of the stria vascularis (Cardinaal et al., 2000; van Ruijven et al., 2005). Several mechanisms including oxidative stress, DNA damage, and inflammatory injury have been suggested to mediate cisplatin ototoxicity. The "traditional" mechanism involves covalent binding of cisplatin to guanine bases on DNA with the subsequent formation of inter- and intra-strand chain cross-linking and the induction of p53, cell cycle arrest, and apoptosis (Cheng et al., 2005; van Ruijven et al., 2005; Zhang et al., 2003). Furthermore, a number of studies demonstrated that reactive oxygen species (ROS) generated by cisplatin increase lipid peroxidation, which alters enzymes and structural proteins, and induces cellular apoptosis (Clerici et al., 1996; Kopke et al., 1997; Rybak et al., 1999a). More recently, it has been shown that the inflammatory pathway is critically involved in cisplatin-induced ototoxicity (Kim et al., 2011b).

Nicotinamide adenine dinucleotide (NAD^+) has emerged as a key regulator of metabolism, stress adaptation, and cellular homeostasis because it acts as a metabolic cofactor and rate-limiting co-substrate for NAD^+-dependent enzymes. Recently, it has been reported that intracellular NAD^+/NADH ratios are decreased in various pathological conditions such as diabetes (Ido, 2007), cisplatin-induced cochlear and kidney damage (Kim et al., 2014b; Oh et al., 2014), and in many tissues of aged animals and humans (Braidy et al., 2011; Massudi et al., 2012). The perturbation of intracellular NAD^+ levels is linked to the progression of various diseases through the production of ROS and inflammation (Kim et al., 2014a, 2014b; Oh et al., 2014). Furthermore, the reduction of the intracellular NAD^+/NADH ratio is critically involved in cisplatin-induced cochlear damage; pharmacologically increasing the cellular NAD^+/NADH ratio suppresses cisplatin-induced cochlear damage by down-regulating potential mediators of cellular damage such as oxidative stress and inflammatory responses (Kim et al., 2014b). The decrease in the NAD^+/NADH ratio has been attributed to hyperactivation of the NAD^+-consuming poly(ADP-ribose) polymerase 1 (PARP-1), which is induced by oxidative damage due to altered redox mechanisms and consequent DNA damage. The silent mating-type information regulation 2 (sirtuins, SIRTs) homologs are NAD^+-dependent deacetylases that are influenced by the NAD^+/NADH ratio. Therefore, a significant reduction in this ratio causes a concomitant decrease in SIRT deacetylase activity, which is critically involved in diverse biological functions (Fig. 1). Therefore, the maintenance of adequate NAD^+ levels may be a critical factor for normal cellular function and could emerge as a useful strategy for treating many diseases. In this review, we describe the mechanisms of cisplatin-mediated ototoxicity and the role of NAD^+ metabolism. Furthermore, we discuss a potential strategy for the prevention of the adverse effects of cisplatin, which involves targeting NAD^+-dependent enzymes, including the SIRTs and PARPs.

2. Biochemical mechanisms of cisplatin ototoxicity

2.1. ROS in cisplatin-induced ototoxicity

ROS including superoxide anions, hydrogen peroxide, and hydroxyl radicals are unavoidable by-products of cellular respiration. A sustained increase in the ROS concentration mainly leads to the death of sensory epithelial cells and, to a lesser extent, innervating neurons (Banfi et al., 2004). In particular, the production of ROS is closely associated with cisplatin-induced ototoxicity (Kim et al., 2014b; Mukherjea et al., 2010; Rybak et al., 2007). The unstable and highly reactive nature of ROS causes them to attack and modify multiple target molecules such as lipids, proteins, and DNA thereby producing cellular stress. ROS also activate important signaling pathways including the apoptotic pathway, which leads to cell death when associated with cisplatin-induced ototoxicity (Kim et al., 2014b; So et al., 2007). Although the role of ROS in cochlear damage is well established, its source is poorly understood. Potential sources of ROS include the mitochondrial electron transport chain system (Szeto, 2006), xanthine oxidase (Berry et al., 2004), cytochrome P450 (CYP) enzymes (Gottlieb, 2003), and NADPH oxidase (Miller et al., 2006). Cisplatin may produce ROS in microsomes via the CYP system. *In vitro* and *in vivo* tests have demonstrated that CYP is an important source of catalytic iron for the generation of ROS during cisplatin treatment. Furthermore, the cisplatin-induced increase in ROS and kidney damage were attenuated in $CYP2E1^{-/-}$ mice (Liu et al., 2003). The mitochondria have also been reported as major sources of ROS. The disruption of the mitochondrial electron transport chain system, which is accompanied by loss of mitochondrial membrane potential, an indicator of mitochondrial dysfunction, is a well-recognized mechanism responsible for the generation of ROS (Szeto, 2006). Interestingly, mitochondria themselves are particularly vulnerable to oxidative stress. Oxidative damage to mitochondria impairs their function and subsequently causes cell death via apoptosis and necrosis (Bai et al., 2001). Thus, ROS-mediated oxidative damage to mitochondria facilitates the generation of additional ROS, resulting in a vicious cycle. In our previous report, we demonstrated that cisplatin-induced mitochondrial membrane potential loss in HEI-OC1 auditory cells was accompanied by the release of cytosolic cytochrome c from the mitochondria (So et al., 2005). These results suggest that mitochondria are important sources of ROS in cisplatin-mediated auditory cell damages. The membrane NAD(P)H oxidases (NOXs) are also one of the major sources of ROS generation. Superoxide is generated by NOX enzyme complexes, especially in phagocytic cells such as neutrophils (Geiszt, 2006). However, many studies have shown that superoxide-generating NOX expression is not restricted to phagocytic cells but is present in a wide variety of non-phagocytic cells and tissues (Krause, 2004; Quinn et al., 2006). In particular, it has been reported that superoxide-generating NOXs are expressed in the inner ear and kidney tissues (Banfi et al., 2004; Kim et al., 2010; Oh et al., 2014). Paffenholz et al. (2004) suggests a physiological role for ROS in the inner ear, in a report showing impaired balance and the lack of otoconia formation in the utricle and saccule of *het* (head tilt) mice, owing to the mutation of NOX3. However, they did not show any gross hearing deficits in the *het* mice, which implies that NOX3 has no overt physiological role in hearing function (Paffenholz et al., 2004). Banfi et al. (2004) demonstrated that cisplatin treatment is associated with increased NOX activity primarily that of NOX3 in the cochlea. They suggested that the induction of NOX3 serves as a major source of ROS generation in the cochlea and forms a part of the pathway leading to cisplatin-mediated damage (Banfi et al., 2004). In addition, it has been demonstrated that NOX1 and NOX4 expression is also increased by exposure to cisplatin, and thereby produce ROS that leads to cisplatin-mediated ototoxicity and nephrotoxicity (Kim et al., 2010; Oh et al., 2014; Pan et al., 2009).