Efficacy of N-acetylcysteine on Prevention and Amelioration of Cisplatin-induced Ototoxicity: A Systematic Literature Review

Maryana Peravozchykava

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EFFICACY OF N-ACETYLCYSTEINE ON PREVENTION AND AMELIORATION
OF CISPLATIN-INDUCED OTOTOXICITY:
A SYSTEMATIC LITERATURE REVIEW

by

MARYANA PERAVOZCHYKAVA

A capstone research project submitted to the Graduate Faculty in Audiology in partial fulfillment of the requirements for the degree of Doctor of Audiology
The City University of New York
2014
This manuscript has been read and accepted for the Graduate Faculty in Audiology in satisfaction of the capstone project requirement for the degree of Au.D.

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Date

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Faculty Mentor

John P. Preece, Ph.D., CCC-A

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Date

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Executive Officer

THE CITY UNIVERSITY OF NEW YORK
Abstract

EFFICACY OF N-ACETYLCYSTEINE ON PREVENTION AND AMELIORATION OF CISPLATIN-INDUCED OTOTOXICITY:
A SYSTEMATIC LITERATURE REVIEW

By
Maryana Peravozchykava

Advisor: Carol A. Silverman, Ph.D., M.P.H.

Objective: The purpose of this investigation is to perform a systematic review of the existing literature on NAC efficacy in prevention of cisplatin-induced ototoxicity.

Methods: A comprehensive search utilizing databases via the Mina Rees Library of the Graduate Center of the City University of New York was conducted to identify relevant studies for analysis. The levels of evidence were applied to restrict articles reviewed to Level 3 or better.

Results: Eight articles evaluating NAC protective efficacy against cisplatin-induced ototoxicity were identified. The results revealed significant variability in NAC otoprotective efficacy.

Discussion: The significant variability in findings on NAC otoprotective efficacy may reflect lack of uniformity in the study methodologies and the limited number of clinical trials. Randomized clinical trials are needed on NAC otoprotective efficacy.

Conclusions: The findings of the reviewed studies show promise in preventing otologic damage with the use of NAC. Nonetheless, based on the currently available evidence, no recommendations for clinical practice can be made at this time.

Key words: N-Acetylcysteine, cisplatin, ototoxicity, otoprotection, sensorineural hearing loss
ACKNOWLEDGMENTS

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I must also thank my fellow students, Brit Boyarski, David Engelman, Talia Meisel, Lillian Law, Ellen May, Theresa Bartoldus, Derek Petti, and especially my studymate and friend Alex Petraru, for always being an incredible source of encouragement and support.

Most importantly, I would like to express my heartfelt gratitude to my family. My family, to whom this capstone is dedicated, has been a constant source of love, concern, support, and strength all these years. I would like to thank my daughter Marcelina and my son Martin, the latest addition to my family, for being my source of joy and my motivating force in all that I do. I am deeply grateful to my husband and best friend, Oleg, for his love, patience and tremendous support throughout my studies and for always believing in me and assisting me in any possible way. I would like to express a special thank you to my mother and my in-laws who have always believed in my potential and encouraged me to achieve my goals.

Thank you all. I would not be where I am today if not for you.
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INTRODUCTION

Ototoxicity is the tendency of certain substances, either systemic or topical, to cause functional impairment and cellular damage to the tissues of the inner ear, particularly to the end organs of the cochlear and vestibular divisions of the eighth cranial nerve (Zarandy & Rutka, 2010). Major pharmacological groups that are ototoxic to humans include the aminoglycosides (e.g., gentamicin, neomycin), platinum-compounds (e.g., cisplatin, carboplatin), loop diuretics (e.g., furosemide, ethacrynic acid), macrolides (e.g., erythromycin), acetylsalicylic acid (aspirin) and non-steroidal anti-inflammatories, topical antiseptics (e.g., chlorhexidine), quinines, iron chelating agents, etc. (Roland & Rutka, 2004).

Cisplatin

Cisplatin, cis-diammine-dichloroplatinum II (CDDP), a platinum-based chemotherapeutic agent that was introduced into clinical chemotherapy in the early 1970s, is the most active platinum compound in experimental tumor systems (Rybak & Whitworth, 2005). It is a highly effective antineoplastic agent that is widely used in the treatment of various malignancies including head and neck cancer, soft-tissue neoplasms, squamous cell cancer, lung cancer, gastric, testicular, bladder, and ovarian cancer. Although cisplatin is one of the most effective chemotherapeutic agents with a cure rate of up to 85%, its clinical use is limited due to severe side effects including ototoxicity, nephrotoxicity, bone marrow toxicity, gastrointestinal toxicity, and peripheral neuropathy (Hartmann & Lipp, 2003b; McKeage, 1995). Nephrotoxicity and ototoxicity side effects of cisplatin represent barriers to the goal of dose escalation of cisplatin to achieve optimal antineoplastic effects. Whereas nephrotoxicity can be ameliorated to some
degree with hydration therapy, ototoxicity still poses a limitation to effective cisplatin chemotherapy (Dickey, Wu, Muldoon, & Neuwelt, 2005).

The incidence of cisplatin ototoxicity ranges from 10% to more than 90% (Helt-Cameron & Allen, 2009; Li, Womer, & Silber, 2004; Skinner, Pearson, Amineddine, Mathias, & Craft, 1990). Cisplatin-induced ototoxicity appears to be dose-dependent, increasing with higher cumulative doses and with higher individual doses (Bertolini, Lassalle, Mercier, Raquin, Izzi, Corradini, & Hartmann, 2004a; Li et al., 2004; Reddel et al., 1982). Bolus injections are more ototoxic than longer infusion durations (Reddel et al., 1982). Additional factors contributing to the variability in reported incidence of cisplatin-induced ototoxicity include differences in ototoxicity criteria and grading systems and in methods used to determine the presence of ototoxicity (e.g., conventional audiometry, high-frequency audiometry, electrophysiological measures) (Roland & Rutka, 2004). Other variables that appear to play a role in the severity of hearing loss following cisplatin administration include the age of the patient (with young children being more susceptible than adults), noise exposure, exposure to other ototoxic drugs, impaired renal function at the time of cisplatin treatment, depleted nutritional state including low serum albumin and anemia, irradiation of the skull base, as well as genetic predisposition (Li et al., 2004; Mukherjea & Rybak, 2011; Roland & Rutka, 2004; Skinner et al., 1990; Weissenstein, Deuster, Knief, Zehnhoff-Dinnesen, & Schmidt, 2012).

Cisplatin-induced ototoxicity is manifested by irreversible and progressive sensorineural hearing loss and/or tinnitus in the high-frequency range. Studies on the long-term effects on hearing, however, have shown that hearing loss increases and also involves the lower frequencies as the toxicity progresses (Einarsson et al., 2010). Cisplatin-induced hearing loss usually is bilateral, and is of heightened concern in the pediatric population, who can incur neuroblastomas,
central nervous system malignancies, and head and neck cancers; and treatment of these lesions often involves irradiation of the skull base (Rybak, Mukherjea, Jajoo, & Ramkumar, 2009).

Although ototoxicity caused by cisplatin may occur within hours to days after drug administration, delayed ototoxicity from cisplatin may occur in children who are particularly susceptible to cisplatin-induced ototoxicity due to their young age (Bertolini, Lassalle, Mercier, Raquin, Izzii, Corradini, & Hartmann, 2004b; Einarsson et al., 2010; Li et al., 2004). A study by Bertolini et al. (2004a) revealed that of the pediatric patients treated with cisplatin, 5% incurred hearing loss before chemotherapy cessation, and 44% incurred significant hearing loss at more than 2 years post chemotherapy cessation. The results of another study on 67 children treated with cisplatin revealed that 61% developed sensorineural hearing loss with the median time to development of hearing loss being 135 days. Additional follow-up for 6 to 44 months post chemotherapy cessation showed further progression of hearing loss by 10 to 15 dB HL up to 26 months post completion of cancer therapy (Knight, Kraemer, & Neuwelt, 2005). The mechanism underlying emergence of hearing loss not only during platinum-based chemotherapy but also years after completion of the chemotherapy might be prolonged retention of platinum in the body; circulating platinum is still detectable in the plasma up to 20 years post treatment (Gietema et al., 2000).

The histopathological otologic degeneration that occurs with cisplatin exposure has been well described (Dickey et al., 2005; Feghali, Liu, & Van De Water, 2001a; Kopke et al., 1997). Multiple studies have shown that cisplatin primarily damages the outer hair cells (OHCs) of the organ of Corti, starting at the cochlear base, and then progressing apically with continued drug exposure. Cisplatin also causes sporadic loss of the inner hair cells (McAlpine & Johnstone, 1990; Poirrier, Pincemail, Van Den Ackerveken, Lefebvre, & Malgrange, 2010; Roland & Rutka, 2000).
Cisplatin toxicity beyond the hair cells includes degeneration of the stria vascularis, collapse of Reissner’s membrane, and damage to the supporting cells in the organ of Corti (Laurell & Bagger-Sjoback, 1991; Ramirez-Camacho, Garcia-Berrocal, Bujan, Martin-Marero, & Trinidad, 2004). All affected cells die predominantly through apoptosis, one of the death pathways a cell follows when it encounters a stress-inducing situation. In a normally functioning cell, a delicate balance of apoptosis-inducing and apoptosis-inhibiting factors exist to ensure that the cell lives and proliferates. In stress-inducing situations, however, this balance is disturbed and through an internal messaging system, the cell may enter the apoptotic death cycle (Hartmann & Lipp, 2003a; McAlpine & Johnstone, 1990).

Although the histopathology of cisplatin ototoxicity has been widely investigated, the molecular mechanisms underlying cisplatin-induced ototoxicity have yet to be fully explained. Accumulation of free radicals (i.e., reactive oxygen species) in the cochlea is postulated to be the main cause of cell death in the inner ear. Reactive oxygen species (ROS), once generated, can react with a variety of cellular components such as protein, DNA, and unsaturated lipids, leading to chemical modification and to metabolic and structural alterations, which, in turn, can lead to cell death. The cochlea, because of its unique anatomical position and isolation, is nearly a closed system. Thus, it cannot flush out accumulated toxins at the rapid pace of their generation, leading to ROS overload and then depletion of the cochlear antioxidant enzyme system that scavenges and neutralizes harmful superoxides (Mukherjea & Rybak, 2011). The cochlear antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione reductase (GSH-R) (Rybak et al., 2009).

Cisplatin chemotherapy not only induces a decrease in the plasma antioxidant levels, which may reflect a failure of the intra and extra cellular antioxidant defense mechanism against
cisplatin-induced oxidative damage, but also suppresses the formation of endogenous antioxidants (Weijl et al., 1998). These conditions lead to the oxidation of biomolecules by ROS, thereby causing irreversible cellular damage. Oxidative damage can manifest in different ways. It can affect the DNA, leading to nucleotide oxidation and ultimately to mutations during the replication process. It can alter protein function by changing the structure and the function of enzymes. Lastly, membrane lipids are preferred targets of oxygen-free radicals and their oxidation can lead to membrane dysfunction and cell destruction. Damage to the mitochondrial membrane results in the release of cytochrome c (usually mediated by Bax, a protein that facilitates apoptotic cell death), which activates caspase 9, and then caspase 3; this process leads to apoptosis of the OHCs (Ciorba, Astolfi, & Martini, 2008; Rybak, Whitworth, & Somani, 1999; Weijl et al., 1998).

**Otoprotection**

Although cisplatin-induced hearing loss is not life-threatening, loss of hearing can have significant consequences for both children and adults. In children, hearing loss can hamper speech, cognitive, and social development with high potential for difficulties in speech discrimination and language acquisition, diminished academic achievement, reduced psychosocial functioning, and diminished quality of life (Gurney et al., 2007; Gurney et al., 2009; Li et al., 2004). In adults, hearing loss has been associated with functional and cognitive impairments that, in turn, can adversely affect mood, communicability, general wellness and routine social interactions essential for independent existence. Untreated hearing loss has also been found to cause feelings of sadness, anxiety, depression, insecurity, and social isolation (Boi et al., 2012; Ciorba, Bianchini, Pelucchi, & Pastore, 2012). Prevention or mitigation of cisplatin-
induced ototoxicity, therefore, may have beneficial effects on quality of life for cancer patients and survivors treated with cisplatin.

In the effort to prevent or mitigate cisplatin-induced ototoxicity, extensive research has been devoted to the identification of medical interventions capable of ameliorating or preventing the adverse effects of cisplatin without compromising its cancer treatment efficacy. Various strategies to counteract cisplatin ototoxicity including oxidative stress-induced apoptosis of the OHCs include the following: (a) preventing the formation of ROS by binding the toxins; (b) reversing toxin binding with cellular proteins, lipids, or DNA; (c) inhibiting the production of extremely toxic lipid peroxidation byproducts, such as 4-hydroxynonenal; (d) adding exogenous free-radical scavengers and antioxidant enzymes and molecules to prevent ROS reactions with cellular proteins, lipids, and DNA; and (e) increasing the activity of the endogenous antioxidant system (Rybak & Kelly, 2003).

Because cisplatin-induced hearing loss involves robust generation of ROS in the cochlea and leads to degeneration of antioxidant system, a basic assumption of treatment strategy is that administration of antioxidants will ameliorate ototoxic effects by preventing the formation of ROS or by allowing clearance of ROS by scavenging mechanisms. According to Theneshkumar and Hatzopoulos (2007), antioxidants providing protection against cisplatin-induced ototoxicity can be classified into the following groups:

1. Compounds that directly scavenge the free radicals formed. Antioxidants chemically bind to the ROS and prevent the reactions of ROS with cellular proteins, lipids, and DNA. These antioxidants are also known as direct antioxidants or chain-breaking antioxidants (e.g., vitamin E, estrogen, salicylate, serotonin, amifostine).
2. Compounds that reduce the formation of free radicals. These compounds chemically bind with the molecules that participate in ROS formation. They are also known as indirect antioxidants (e.g., iron chelators, calcium antagonists, glutamate receptor antagonists).

3. Compounds that support the endogenous antioxidant production - these compounds participate in endogenous antioxidant production or in antioxidant recycling (e.g., N-acetylcysteine, D-methionine and lipoic acid) (Theneshkumar & Hatzopoulos, 2007).

A number of promising otoprotective agents are in preclinical and clinical development. The challenges lie in selecting the best products for further investigation, evaluating their efficacy and safety, and introducing them into clinical practice. According to Brock et al. (2012), the ideal otoprotectant is effective (reliable otoprotection), safe (no tumor preservation), has minimal adverse effects, uses simple administration techniques, is suitable for use with various platinum compounds and schedules of administration, and is of sufficient interest to the pharmaceutical industry for investment in research and development.

Currently, no pharmacologic agent has United States Food and Drug Administration approval to prevent or reverse platinum-induced hearing loss. The most promising results have been demonstrated using thiol compounds and antioxidants. Sodium thiosulfate, which is among the most widely studied of the thiol compounds, repeatedly has demonstrated cisplatin otoprotection in animal and clinical studies (Stocks et al., 2004; Wang et al., 2003). D-Methionine and L-Methionine are other thiol compounds that have been investigated with similar success when given systemically (Campbell, Rybak, Meech, & Hughes, 1996; Campbell et al., 2007; Gopal et al., 2012; Korver, Rybak, Whitworth, & Campbell, 2002). The main limitation of
systemic thiol use for cancer patients to prevent or reverse cisplatin-induced hearing loss, however, is the associated marked decrease in the oncologic effectiveness of cisplatin (Dickey et al., 2005; Ekborn et al., 2002). Other routes of drug administration have been considered, including intratympanic injection (IT) and round window membrane instillation. IT injection is a viable option in the clinical setting that allows local administration of medication with the possibility of minimal systemic diffusion. Chemicals inserted into the middle ear have to cross the round window membrane to reach the target cells in the inner ear. Characteristics that determine the ease with which a molecule will cross the round window membrane include its molecular weight, electric charge, and liposolubility (Nader, Theoret, & Saliba, 2010).

N-Acetylcysteine (NAC), a well-known pharmaceutical agent, is another thiol-containing agent with promising otoprotective efficacy. NAC, a strong free radical scavenger and a precursor of glutathione, is an antioxidant that limits the extent of the oxidative stress damage to the cell and is able to improve the oxidant/antioxidant cellular balance (Atkuri, Mantovani, Herzenberg, & Herzenberg, 2007; Lorito et al., 2011). Of importance, NAC is well-established as being safe and effective in systemic use for treatment of acetaminophen poisoning. Its safety profile also is well established for mucosal surfaces as a mucolytic agent for chronic bronchitis and as eye drops for keratoconjunctivitis sicca (Atkuri et al., 2007; Holdiness, 1991). At the present time, NAC appears to be the most promising thiol-containing medication for human applications (Yoo et al., 2013). NAC may protect the inner ear from the ototoxic effects of cisplatin both by acting as a free radical scavenger, and by inducing the production of endogenous antioxidants (van den Berg, Beijnen, Balm, & Schellens, 2006). Its protective properties against cisplatin seem to apply both to the OHCs and auditory neurons (Feghali, Liu, & Van De Water, 2001b). In addition, due to its low molecular weight of 163.2Da, NAC is an
excellent candidate for administration across the round window and it allows for high cochlear concentration with negligible systemic absorption (Choe, Chinosornvatana, & Chang, 2004).

The primary purpose of this investigation is to perform a systematic review of the existing literature on NAC efficacy in prevention or amelioration of cisplatin-induced ototoxicity. Towards this goal, the effects of NAC dose and route of administration on prevention or amelioration of cisplatin-induced hearing loss will be examined. Also, any adverse effects of NAC on cisplatin anti-tumor efficacies and possible side effects of NAC administration will be identified.
METHODS

Studies evaluating cisplatin-based chemotherapy together with NAC intervention versus cisplatin-based chemotherapy with placebo, with no additional treatment, or with another protective intervention in animal or human subjects were reviewed.

Database Search

Comprehensive searches utilizing databases via the Mina Rees Library of the Graduate Center of The City University of New York were performed to identify relevant publications to be included in this review. A global search was performed between the dates of September, 2012 to October, 2012; the most recent search was accomplished in October, 2013 for the purpose of identification of the latest publications. The search for relevant literature took place on the title, abstract and full-text levels throughout the electronic and manual database search. The following databases were employed to search for relevant studies: Academic Search Complete, EbscoHost, Science Direct, PubMed Central, Scopus, and Google Scholar. Some articles not available directly via the Mina Rees Library were requested through the Interlibrary Loan. Additionally, when an article was found to be relevant to the topic of interest, the article’s reference list was searched for relevant studies not identified in the direct database search. The search was limited to articles published in English and Russian.

To identify pertinent studies in the existing literature a search was conducted using various combinations of the following key words: N-acetylcysteine, L-N-acetylcysteine, N-acetyl-L-cysteine, Acetyl Cysteine, cisplatin, cis-dichlorodiammineplatinum, cis-dichlorodiammineplatinum II, cisplatin-induced, ototoxicity, cochleotoxicity, hearing, hearing loss, hearing impairment, otoprotection, and protection. The key words "cisplatin" and "N-Acetylcysteine" were used consistently throughout the search. The terms "nephrotoxicity", "
"aminoglycosides", “carboplatin”, and “noise-induced” were at times included in the search strings using Boolean operator "NOT" to limit retrieval of irrelevant studies. No limiters were applied for date of publication.

**Evaluation Procedure to Limit Articles Reviewed**

A rating of strength of evidence was applied to the body of research reviewed. The rating system used was based on Cox’s (2005) description of levels of evidence. Level 1, the highest level of evidence, comes from systematic reviews and meta-analyses of randomized controlled trials or other high-quality studies. Level 2 evidence comes from randomized controlled trials such as those more commonly seen in drug or other treatment efficacy studies. Level 3 studies involve intervention, but the methods do not include random assignment of participants to treatment groups. Level 4 studies do not include a treatment or intervention. These studies involve the observation of a group or groups of subjects with a given condition or treatment over time. Level 5 evidence comes from reports of individual cases with given conditions or treatments. Lastly, Level 6 evidence comes from expert opinion based on experience and/or knowledge of the subject. Cox’s levels of evidence were applied to restrict articles reviewed to Level 3 or better studies.

**Types of Outcome Measures Reviewed**

Hearing loss and/or tinnitus represent primary outcomes (as defined by the authors of the original studies) of cisplatin treatment (in conjunction with NAC administration) that were reviewed.

The following (any one or combination) represent secondary outcomes (as defined by the authors of the original studies) of cisplatin treatment (in conjunction with NAC administration)
that were reviewed: (a) tumor response (complete or partial remission); (b) survival (overall survival and event-free survival); (c) adverse effects other than hearing loss and tinnitus.
RESULTS

The results of the database search are shown in Figure 1.

Potentially relevant publications identified through electronic database searching using keywords: N = 304

Publications excluded from review based on irrelevant title: N = 213

Publications, excluding duplicates, assessed for eligibility based on abstract: N = 31

Publications excluded following abstract review due to irrelevant tumor therapy or ototoxicity intervention: N = 17

Publications excluded following full article review due to irrelevant tumor therapy or ototoxicity intervention: N = 6

Publications included following manual search of reference lists of included studies: no additional studies identified.

Publications included for review: N = 8

Figure 1. Database search and retrieval process
Running the searches in the electronic databases yielded a total of 304 references. Exclusion of irrelevant articles and duplicated articles yielded 31 articles for abstract review. Abstract review of 31 articles resulted in the exclusion of an additional 17 articles as they did not appear to be relevant to the study topic. Full article review of the remaining 14 identified articles was necessary to determine pertinence to this review. Based on the full article review, eight articles received full evaluation. Manual search of the reference lists of publications failed to identify any additional eligible studies.

Of the 8 studies, 63% demonstrated level 3 evidence (they were non-randomized control trials) and 37% (Choe et al., 2004; Nader et al., 2010; Riga et al., 2013) demonstrated level 2 evidence as they were randomized control trials. All studies were prospective and described study rationale, criteria for inclusion or exclusion of subject, procedures, and outcome measure(s).

**Subject Characteristics**

As shown in Table 1, of the 8 studies included in this review, 37% involved human participants and 63% involved animal subjects. Of the 5 animal studies, 40% were studies on rats and 60% were studies done on guinea pigs (see Table 1). The gender of the animals was male in the Lorito et al. (2011) study, female in the Dickey et al. (2004) study, and was unspecified in the remaining studies on guinea pigs.

The number of participants that completed each study is specified in the Table 2. In the Riga et al. (2013) study, 20 (5 females and 15 males, 16 to 77 years of age) of the 24 initially enrolled patients were eligible for evaluation. One patient was excluded from the study after developing middle-ear infection following the second intratympanic injection of NAC, and three patients were denied further treatment due to acute pain in the middle ear that developed.
immediately after the infusion. Of the 13 initially enrolled in the study by Yoo et al. (2013), 11 male patients (29 to 68 years of age) were eligible for evaluation; two patients withdrew from the study, 1 patient required hospitalization prior to initiation of cancer treatment and subsequently withdrew from the study because he felt too ill to participate, and another patient withdrew from the study prior to initiation of any intervention for unspecified reason. Since only male subjects completed that study, the results obtained may not be generalizable to females. In the Yildirim et al. (2010) study, all of 54 participants (28 females and 26 males, 29 to 71 years of age) who met the study criteria completed the investigation. Additionally, two animals were excluded from the study by Choe et al. (2004) because of poor baseline distortion product otoacoustic emissions (DPOAEs). In the remaining investigations, as shown in Table 2, no enrolled participant withdrew before the study was completed.

The participants of the human studies presented as heterogeneous groups with respect to age. The study sample size varied substantially, ranging from 13 to 54 in the human studies and from 10-53 in the animal studies (see Table 1). The mortality rate, resulting from cisplatin toxicity, was 30% and 7.5% in the Saliba et al. (2010) and Choe et al. (2004) studies, respectively.

Table 1

*Study Findings including Type of Subject, Drug Intervention¹, Drug Delivery Route², and Drug Dose*
<table>
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<tr>
<th>Study</th>
<th>Type</th>
<th>Sample size</th>
<th>Group</th>
<th>Route of delivery</th>
<th>Individual dose</th>
<th>Total dose</th>
<th>Route of delivery</th>
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<tbody>
<tr>
<td>Riga et al. (2013)</td>
<td>Human</td>
<td>N = 24</td>
<td>N/A</td>
<td>IV</td>
<td>50-100 mg/m²</td>
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<td>Human</td>
<td>N = 13</td>
<td>N/A</td>
<td>IV</td>
<td>100 mg/m²</td>
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<td>Lorito et al. (2011)</td>
<td>Sprague-Dawley rats</td>
<td>N = 40 (N = 4 per group)</td>
<td>3 groups: D-Met (groups differed in dose)</td>
<td>3 groups: NAC (groups differed in dose)</td>
<td>3 groups: Ebselen (groups differed)</td>
<td>IV</td>
<td>14 mg/kg</td>
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<tr>
<td>Nader et al. (2010)</td>
<td>Hartley guinea pigs</td>
<td>N = 16 (N = 8 per group)</td>
<td>NAC Lactate</td>
<td>IP</td>
<td>3 mg/kg weekly</td>
<td>24 mg/kg</td>
<td>IT</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Yildrim et al. (2010)</td>
<td>Human</td>
<td>N = 54 (N = 18 per group)</td>
<td>NAC with Cis Salicylate with Cis Control</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>600 mg/day</td>
<td>Not specified</td>
</tr>
<tr>
<td>Saliba et al. (2010)</td>
<td>Guinea pigs</td>
<td>N = 10 (N = 5 per group)</td>
<td>NAC with Cis MP with Cis</td>
<td>IP</td>
<td>3 mg/kg</td>
<td>15 mg/kg</td>
<td>IT</td>
<td>~0.2 ml</td>
<td>?</td>
</tr>
<tr>
<td>Choe et al.</td>
<td>Guinea pigs</td>
<td>N = 53 (N = 13)</td>
<td>NS</td>
<td>IP</td>
<td>10 mg/kg</td>
<td>20 mg/kg</td>
<td>IT</td>
<td>~0.2 ml</td>
<td>~ 0.2 ml</td>
</tr>
<tr>
<td>Study (2004)</td>
<td>Type</td>
<td>Sample size</td>
<td>Subjects</td>
<td>Cisplatin intervention</td>
<td>NAC intervention</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>------</td>
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<td>------------------------</td>
<td>------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dickey et al. (2004)</td>
<td>Rat</td>
<td>N = 30</td>
<td>NAC (N = 14)</td>
<td>LR (N = 14)</td>
<td>Control (N = 12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NAC 15 min prior to Cis (N = 8)</td>
<td>NAC 30 min prior to Cis (N = 7)</td>
<td>NAC 4 hours post Cis (N = 7)</td>
<td>Saline 15 min prior to Cis (N = 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IA 6 mg/kg</td>
<td>IA 6 mg/kg</td>
<td>IV 400 mg/kg</td>
<td>IV 400 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1D-methionine (d-Met); N-acetyl cystein (NAC); cisplatin (Cis); methylprednisolone (MP); lactated Ringer’s solution (LR); normal saline (NS).

2Intratympanic (IT); intraperitoneal (IP); intravenous (IV); intra-arterial (IA).

Table 2

*Number of Subjects Completing the Study, NAC Efficacy, Cis Ototoxicity, and Cancer (Ca) Treatment (Tx) Compromise by NAC*

<table>
<thead>
<tr>
<th>Study</th>
<th>Evaluated n/N (%)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>NAC efficacy +/-&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Cis ototoxicity +/-&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Ca tx Compromise by NAC +/-&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riga et al. (2013)</td>
<td>20/24 (84%)</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Yoo et al. (2013)</td>
<td>11/13 (85%)</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Lorito et al. (2011)</td>
<td>40/40 (100%)</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Nader et al. (2010)</td>
<td>16/16 (100%)</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Yildrim et al. (2010)</td>
<td>54/54 (100%)</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Saliba et al. (2010)</td>
<td>7/10 (70%)</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Choe et al. (2004)</td>
<td>47/53 (88.7%)</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Study</td>
<td>Evaluated n/N (%)</td>
<td>NAC efficacy +/-</td>
<td>Cis ototoxicity +/-</td>
<td>Ca tx Compromise by NAC +/-</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------</td>
<td>-----------------</td>
<td>---------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Dickey et al. (2004)</td>
<td>30/30 (100%)</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
</tbody>
</table>

1 n/N indicates number of subjects who completed the study as compared with the number of subjects who initially enrolled in the study.

2“+” indicates drug was efficacious; “-“ indicates drug was not efficacious.

3“+” indicates drug has ototoxic effects; “-“ indicates absence of ototoxic effect.

4“+” indicates efficacy of cancer treatment was compromised by NAC; “-“ indicates efficacy of cancer treatment was not compromised by NAC.

**Cisplatin Intervention**

As shown in Table 1, intravenous (IV) cisplatin infusions were administered in three studies (Lorito et al., 2011; Riga et al., 2013; Yoo et al., 2013). Cisplatin delivery was by intraperitoneal (IP) injection in three studies (Choe et al., 2004; Nader et al., 2010; Saliba et al., 2010), and by intra-arterial (IA) injection in one study (Dickey et al., 2004). The route of cisplatin administration was unspecified in one study (Yildirim et al., 2010).

Individual and cumulative cisplatin dose varied considerably among the studies (see Table 1). In the studies on human participants, individual cisplatin dose ranged from 50 to 100 mg/m² of body surface area. In the animal studies, individual dose ranged from 3-14 mg/kg. Whenever specified, the cumulative dose ranged from 120-720 mg/m² in the studies on human participants and from 6 to 24 mg/kg in the animal studies. In the Yoo et al. (2013) study, the total
number of cisplatin cycles ranged from 2 to 6, which yields a cumulative dose between 200 and 600 mg/m². Yildrim and colleagues (2010), who employed only one round of cisplatin administration, did not specify either the individual or cumulative dose.

Ototoxicity is one of the well-known adverse effects of cisplatin chemotherapy. Other known side effects of cisplatin intervention include nephrotoxicity, gastrointestinal toxicity, bone marrow suppression, and peripheral neuropathy (Dickey et al., 2004). Table 3 shows the adverse effects that have been identified in the reviewed studies. Half of the studies either did not investigate side effects of cisplatin therapy or did not report them. In the remaining half of the studies, the rates of mortality and weight loss have been equally reported as side effects of cisplatin administration.

Table 3

*Adverse Effects of Cisplatinum-Based Chemotherapy and NAC Intervention*

<table>
<thead>
<tr>
<th>Study</th>
<th>Side effects of cisplatin</th>
<th>Side effects of NAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riga et al. (2013)</td>
<td>Unspecified</td>
<td>Acute pain in the middle ear</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle ear infection</td>
</tr>
<tr>
<td>Yoo et al. (2013)</td>
<td>Unspecified</td>
<td>No incidence of local toxicity or pain</td>
</tr>
<tr>
<td>Lorito et al. (2011)</td>
<td>Weight loss</td>
<td>Unspecified</td>
</tr>
<tr>
<td>Nader et al. (2010)</td>
<td>Unspecified</td>
<td>Inflammation of middle ear mucosa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diffuse osteitis</td>
</tr>
<tr>
<td>Yildrim et al. (2010)</td>
<td>Unspecified</td>
<td>Unspecified</td>
</tr>
<tr>
<td>Saliba et al. (2010)</td>
<td>30% mortality rate</td>
<td>Inflammation of the external auditory canal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inflammation of middle ear mucosa</td>
</tr>
<tr>
<td>Choe et al. (2004)</td>
<td>7.5% mortality rate</td>
<td>Unspecified</td>
</tr>
<tr>
<td>Dickey et al. (2004)</td>
<td>Weight loss</td>
<td>Unspecified</td>
</tr>
</tbody>
</table>
NAC intervention

NAC concentration, route, timing of administration, and dosage are shown in Table 4. In 63% of the 8 studies, NAC solution was injected intratympanically (Choe et al., 2004; Nader et al., 2010; Riga et al., 2013; Saliba et al., 2010; Yoo et al., 2013). The predominance of IT route of administration can be explained by the fact that IT injection has been hypothesized to be an optimal administration route, capable of minimizing both the interference with the antitumor effectiveness of cisplatin and the adverse effects from the systemic administration of the otoprotective agent (Lorito et al., 2011). NAC was administered as a bolus IP injection in one study (Lorito et al., 2011) and as an IV infusion also in one study (Dickey et al., 2004).

Table 4

NAC Concentration, Route, Administration Regimen, and Dosage

<table>
<thead>
<tr>
<th>Study</th>
<th>NAC concentration</th>
<th>Delivery route</th>
<th>Timing of administration</th>
<th>Dose</th>
<th>Number of infusions</th>
<th>Cumulative dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riga et al. (2013)</td>
<td>10%</td>
<td>IT</td>
<td>Prior (exact time not specified)</td>
<td>0.4 – 0.8 ml</td>
<td>3 – 7</td>
<td>?</td>
</tr>
<tr>
<td>Yoo et al. (2013)</td>
<td>2%</td>
<td>IT</td>
<td>30-60 minutes prior</td>
<td>2 – 3 ml</td>
<td>2 – 6</td>
<td>?</td>
</tr>
<tr>
<td>Lorito et al. (2011)</td>
<td>?</td>
<td>IP</td>
<td>1 hour prior</td>
<td>Varied by group: 275, 375, &amp; 475 mg/kg</td>
<td>1</td>
<td>Varied by group: 275, 375, &amp; 475 mg/kg</td>
</tr>
<tr>
<td>Nader et al.</td>
<td>20%</td>
<td>IT</td>
<td>1 hour prior</td>
<td>?</td>
<td>8 times, once a</td>
<td>?</td>
</tr>
</tbody>
</table>
As illustrated in Table 4, NAC concentration varied significantly, from 2% to 20%, independent of whether human or animal subjects were under investigation. Low NAC concentrations were chosen based on clinical safety, since concentrations of NAC higher than 2% have been reported to induce an inflammatory response in the middle-ear mucosa of animals (Choe et al., 2004; Yoo et al., 2013). Despite the reports of substantial middle-ear inflammations caused by high NAC concentration, the efficacy of higher NAC concentrations was investigated (Nader et al., 2010; Riga et al., 2013; Saliba et al., 2010). Because the round window membrane and the middle-ear mucosa are much thinner in animals than in humans, the NAC concentrations in the human study by Riga et al. were much higher (10% and 20%) than the one reported to
cause inflammatory response. Ultimately, the 20% NAC concentration was deemed to be inappropriate for IT infusions after its use in five patients who were eventually excluded from the study because of the development of middle ear and tympanic-membrane inflammation along with strong acute pain. Three studies did not specify the NAC concentration (Dickey et al., 2004; Lorito et al., 2011; Yildirim et al., 2010).

All the investigators of the reviewed studies administered NAC prior to cisplatin chemotherapy with the exception of one study (Dickey et al., 2004), in which NAC was administered 15 minutes and 30 minutes prior to cisplatin injection to two separate groups, and at 4 hours post cisplatin injection to another group. The timing of NAC administration ranged from 30 - 60 minutes in half of the studies reviewed (Lorito et al., 2011; Nader et al., 2010; Saliba et al., 2010; Yoo et al., 2013). In the studies that examined NAC efficacy in human subjects, NAC administration corresponded with the number of chemotherapy treatment cycles, ranging from 3 to 7 (Riga et al., 2013) and from 2 to 6 infusions (Yoo et al., 2013). Although IT NAC injections were performed in both of these studies, the dose was significantly higher in the study by Yoo et al. (2 – 3 ml) than in the study by Riga and colleagues (0.4 – 0.8 ml). The otoprotective effect of 0.2 ml of NAC was examined in two animal studies (Choe et al., 2004; Saliba et al., 2010). In one animal study (Saliba et al., 2010), the number of NAC injections corresponded to the number of cisplatin infusions necessary to reach the cumulative cisplatin dose that is required to induce ototoxicity. A similar protocol for NAC administration was employed in another study (Nader et al., 2010) except that the weekly NAC administrations occurred for eight rather than five consecutive weeks. In the remaining studies, NAC administration was limited to a single injection (Choe et al., 2004; Dickey et al., 2004; Lorito et al., 2011; Yildirim et al., 2010). In two animal studies with single NAC injection, the dose ranged from 275 mg/kg to 475 mg/kg
(Dickey et al., 2004; Lorito et al., 2011) and in one human study the dose was 600 mg/day. The efficacy of various doses of NAC as an otoprotective agent in the animal model was investigated only in one study (Lorito et al., 2010).

**Ototoxicity Assessment**

Conventional pure-tone audiometry (see Table 5) was employed in two (Riga et al., 2013; Yoo et al., 2013) of the three human trials, with high frequency audiometry (HFA) also utilized in one of the studies (Yoo et al.). In another study on human subjects, HFA was employed in addition to auditory brainstem response (ABR) testing with click stimuli (Yildirim et al., 2010). In all of the animal studies, ABR testing was utilized to establish threshold change resulting from ototoxicity, with the exception of one study (Choe et al., 2004), that employed DPOAE testing as the only method of ototoxicity assessment. In the Lorito et al. (2010) study, DPOAE testing was used in addition to ABR testing, but the frequency range examined varied from 6000-18000 Hz as opposed to the 2000 – 16000 Hz range used in the Choe et al. (2004) study. The tonal ABR stimuli used in the animal studies ranged from 1000 to 20000 Hz, with 8000 Hz used in all of these studies. Besides electrophysiologic measures, histopathological analysis of the cochlea using electron microscopy was used in 2 (Nader et al., 2010; Saliba et al., 2010) of the 5 animal studies.

Table 5

<table>
<thead>
<tr>
<th>Study</th>
<th>Ototoxicity assessment</th>
<th>Frequency (Hz), if applicable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riga et al. (2013)</td>
<td>Conventional pure tone audiometry</td>
<td>250, 500, 1000, 2000, 4000, 8000</td>
</tr>
<tr>
<td>Study</td>
<td>Ototoxicity assessment</td>
<td>Frequency (Hz), if applicable</td>
</tr>
<tr>
<td>----------------------</td>
<td>------------------------</td>
<td>--------------------------------</td>
</tr>
</tbody>
</table>
| Yoo et al. (2013)    | Conventional pure tone audiometry | 250, 500, 1000, 2000, 3000, 4000, 6000, 8000  
|                      | HFA                    | 9000, 10000, 11200, 12500, 14000, 16000, 18000, 20000 |
| Lorito et al. (2011) | ABR                    | 8000, 12000, 16000 |
|                      | DPOAE                  | 6000, 8500, 10000, 14000, 18000 |
| Nader et al. (2010)  | ABR                    | 2000, 4000, 6000, 8000 |
|                      | Electron microscopy    | N/A                                    |
| Yildrim et al. (2010)| HFA                    | 10000 and 12000¹ |
|                      | ABR                    | Click                                  |
| Saliba et al. (2010) | ABR                    | 1000, 2000, 3000, 4000, 6000, 8000 |
|                      | Electron microscopy    | N/A                                    |
| Choe et al. (2004)   | DPOAE                  | 2000, 2378, 2828, 3364, 4000, 4757, 5657, 6727, 8000, 9514, 11314, 13454, 16000 |
| Dickey et al. (2004) | ABR                    | 4000, 8000, 12000, 16000, 20000 |

¹ Threshold shift at other frequencies also was evaluated, but only data for 10000 and 12000 Hz are shown because statistical significance was obtained only at these frequencies.

### Cisplatin Efficacy

Ototoxicity resulting from cisplatin-based chemotherapy was reported in all of the studies (see Table 2). The same does not apply with regards to the efficacy of cisplatin intervention in conjunction with NAC administration (see Table 2), which is a crucial outcome measure for cancer treatment. Considering that the efficacy of cisplatin-based chemotherapy is judged by cancer responsiveness to treatment, this efficacy can be established only in the human trials; none of the animal studies had animals with cancer. Thus, only 38% of the eight studies potentially could be examined to determine efficacy of cisplatin therapy. But efficacy of cisplatin...
was in fact examined in only 1 (Riga et al., 2013) of those 3 studies; in that study, complete 
response to cisplatin therapy was achieved in 35%, partial response was achieved in 35%, stable 
disease was seen in 10%, and disease progression was observed in 15%. The observed response 
to chemotherapeutic intervention indicates that cisplatin-based therapy was not substantially 
compromised by NAC administration. To investigate the possibility of NAC interaction with the 
chemotherapy medication, which can possibly result in the deactivation of the latter, high-
performance liquid chromatography (HPLC) was utilized in one animal study (Nader et al., 
2010). The purpose of HPLC was to determine whether NAC is absorbed systemically when 
administered locally (IT) as systematic absorption can possibly interfere with cisplatin efficacy. 
The data obtained by Nader and colleagues did not show any systemic absorption of NAC at 30 
minutes up to three hours after IT injection. Therefore, this finding suggests that the drug 
interaction, which can possibly cause reduction in cisplatin efficacy, is unlikely.

**NAC Efficacy**

Significant variability in study methodology (specifically NAC dosage and concentration, 
cisplatin dosage and administration regimen) complicates the comparative analysis of NAC 
efficacy. Despite the study differences in ototoxicity assessment, frequency of the observed 
effect, NAC and cisplatin administration protocols, all of the examined studies demonstrated 
complete or partial otoprotection by NAC, with the exception of the study by Nader et al. (2010) 
(see Table 2). Nader and colleagues observed a significant increase in ABR thresholds even after 
giving guinea pigs 9 mg/kg of cisplatin, a level that is not considered to be ototoxic (as cited by 
Nader et al.). In the Nader et al. study, 20% NAC solution was administered intratympanically to 
one ear, with the contralateral ear serving as a control. The mean threshold increase of 81.5 dB 
(SD = ± 8.4 dB) was observed in the NAC treated ears only and was equivalent across frequency;
the threshold increases were attributed to middle and inner ear inflammatory responses caused by a high NAC concentration in the solution.

In another study (Riga et al., 2013) the use of 20% NAC solution was attempted in five human subjects; however, the efficacy of high NAC concentration could not be established since those patients were excluded from the results of the study due to development of middle ear and tympanic membrane inflammation in addition to intolerable acute pain (Riga et al., 2013). Nonetheless, 10% NAC solution administered intratympanically was found to be partially efficacious. The results revealed that the pure-tone air-conduction thresholds at one month post chemotherapeutic intervention did not differ significantly from the pre-intervention thresholds in the NAC treated ears; in the control ears, the post chemotherapeutic thresholds were significantly higher than the pre-chemotherapeutic thresholds at 8000 Hz. The protective effects of NAC in human subjects also have been assessed in the study by Yildrim et al. (2010). In that study, the pure-tone air- and bone-conduction thresholds revealed significant differences at 10000 and 120000 Hz between the NAC treatment and control groups at 38 to 42 days post cisplatin exposure; no group differences were observed, however, on the ABR threshold results. The description of the NAC and cisplatin protocols was limited to specification only of NAC dose (600 mg/day). Therefore, based on that study, conclusions regarding the NAC concentration that is partially protective against cisplatin-induced hearing loss cannot be drawn.

Yoo et al.’s (2013) findings failed to demonstrate significant otoprotection with 2% NAC solution. Furthermore, at one to two months post cisplatin treatment, no significant differences between the NAC and control ears were found in the pure-tone air-conduction thresholds, word recognition score, or subjective tinnitus. Nonetheless, IT NAC was associated with better air-conduction pure-tone audiometric thresholds in 2 of the 11 participants studied. The findings in
those two participants were compelling, as the pure-tone average (the authors based this average on 2000, 4000 and 8000 Hz) in the NAC-treated ear was better than that in the control ear by 18-28 dB. The authors attributed the otoprotective effect to NAC administration. Interestingly, those two participants seemed more susceptible to hearing loss at the pure-tone average frequencies in the control ears, as compared to the participants who did not demonstrate a significant effect with NAC.

Partial (with regards to frequency) threshold preservation of the DPOAEs in guinea pigs treated with 2% NAC solution was reported by Choe et al. (2004). Comparison of NAC-treated ears to contralateral control ears indicated significant threshold preservation at 4757, 9514, 11314, and 13454, and particularly at 16000 Hz. Since ABR testing was not performed in this study, a physiologic estimate of the amount of threshold preservation could not be established.

An attempt to assess dose-dependent efficacy of NAC with respect to otoprotection was done in one study (Lorito et al., 2011). Three different NAC doses (275, 375, and 475 mg/kg) were administered to rats via IP injection. Significant decreases in DPOAE amplitude following 14 mg/kg of cisplatin chemotherapy were noted in all groups independent of NAC dosage, suggestive of the lack of an otoprotective effect for NAC. Conversely, the ABR data from the NAC-treated rats showed partial auditory threshold preservation for the two lowest doses (i.e., 275 and 375 mg/kg) and complete threshold preservation for the highest dose (475 mg/kg). Specifically, no significant ABR threshold change post cisplatin administration was observed at 8000 Hz for all three doses, whereas only the NAC group receiving the 475 mg/kg dose presented post-treatment thresholds comparable to pre-treatment thresholds at 12000 and 16000 Hz. The observed discrepancy between the ABR and DPOAE findings was attributed to the minor loss of OHCs in the treated animals, which was manifested in the DPOAE alterations.
Thus, the investigators concluded that the NAC protocols do not offer complete auditory preservation for doses below 400 mg/kg.

Similar findings were reported by Dickey et al. (2004) who observed that a pre-treatment NAC injection of 400 mg/kg NAC prevented ototoxicity in rats; however, the cisplatin cumulative dose was significantly lower (6 mg/kg) in this than in the Lorito et al. (2011) study. Based on the ABR threshold data, NAC was found to be significantly protective against cisplatin-induced ototoxicity for NAC administration at 15 and 30 minutes before and at 4 hours post cisplatin injection.

Saliba et al.’s (2010) findings on NAC efficacy were inconclusive. In guinea pigs who received IT injection of 4% NAC solution prior to cisplatin intervention, they observed a statistically significant mean ABR threshold shift of 19 dB. Nonetheless, similar findings were observed in the control ears of the NAC-treated animals, raising an important question about the preservation of the antineoplastic quality of cisplatin after NAC administration. Comparison of these threshold shifts with those for with the group treated with methylprednisolone revealed that NAC-treated group exhibited smaller threshold shifts than the methylprednisolone treated group. Saliba and colleagues hypothesized that the observed decreased ototoxicity in the NAC group, both in the injected and control ears, might be explained by the fact that NAC passed through the bloodstream and induced an overall reduction in cisplatin ototoxicity and perhaps its efficacy. Cisplatin’s ototoxic effect is not questioned since evidence of ototoxicity was observed in another group with the same cisplatin protocol but with injection of a different ototprotective agent. Thus, the Saliba et al. investigation appears to be the only one of eight reviewed in which drug interaction was suspected, although it was not specifically investigated.
Besides NAC otoprotective efficacy against cisplatin-induced ototoxicity, a majority of the reviewed studies (5) investigated the adverse effects of NAC intervention (see Table 3). In 80% of the 5 studies on NAC side effects, an inflammatory response of the middle ear mucosa was observed (Choe et al., 2004; Nader et al., 2010; Riga et al., 2013; Saliba et al., 2010). In the Riga et al. investigation, acute pain in the middle ear immediately following NAC injection was reported by all study participants, in contrast with the absence of reported pain or local toxicity associated with NAC infusion in the Yoo et al. (2013). Nader et al. reported diffuse osteitis in all NAC-treated ears; and, as previously mentioned, Saliba et al. (2010) observed an inflammatory response in the external auditory canal following NAC injection.
DISCUSSION

The primary purpose of this investigation was to perform a systematic review of the existing literature on NAC efficacy in prevention or amelioration of cisplatin-induced ototoxicity. Towards this goal, the effects of NAC dose and route of administration on prevention/amelioration of cisplatin-induced hearing loss were examined. Also, any adverse effects of NAC on cisplatin anti-tumor efficacies and possible side effects of NAC administration were identified.

Cisplatin-based therapy is currently the most effective form of chemotherapy for treatment of a wide number of adult and pediatric malignancies. Unfortunately, one of the most common and major adverse effects to cisplatin therapy is the occurrence of ototoxicity that both restricts treatment protocols and reduces the quality of life. It currently is the primary dose-limiting factor in cisplatin therapy (Dickey et al., 2005). Thus, prevention of cisplatin-induced ototoxicity is very important for optimal outcomes of cisplatin therapy.

To adequately ascertain the efficacy of an otoprotective medical intervention the best study design, provided that the design and execution are correct, is a randomized controlled trial in which the only difference between the intervention group and control group is the use of the otoprotective agent. Controlled clinical trials also can provide reliable information, keeping in mind their limitations (van den Berg et al., 2006). Eight studies evaluating the use of NAC versus the use of either no otoprotection or different otoprotective agent were identified; of these 8, 3 were randomized controlled trials and 5 were controlled clinical trials. The investigators of the reviewed studies tested the hypothesis that treatment with the thiol agent NAC would reduce or ameliorate ototoxicity of cisplatin in human and animal subjects. Since NAC is relatively
inexpensive, its successful implementation as otoprotective treatment would be highly cost-effective in comparison with management with hearing aids or cochlear implants (Riga et al., 2013; van den Berg et al., 2006).

Dickey et al. (2004) presented evidence that IV treatment with 400 mg/kg NAC at 15 or 30 minutes prior to IA administration of 6 mg/kg cisplatin can prevent cisplatin-induced ototoxicity. Similar findings were presented by Lorito et al. (2011), who reported complete auditory preservation by IP administration of 475 mg/kg NAC at 1 hour prior to the IV administration of 14 mg/kg of cisplatin. Riga et al. (2013) achieved auditory preservation at 8000 Hz with IT administration of 0.4-0.8 ml NAC following IV administration of 120-720 mg/m² cisplatin. Similarly, Yildrim et al. (2010) observed threshold preservation at 10000 and 12000 Hz post 600 mg/day NAC intervention. Choe et al. (2004) observed partial preservation of the DPOAEs following IT administration of 0.2 ml NAC prior to IP administration of 20 mg/kg cisplatin. The remaining studies reviewed failed to report NAC otoprotective efficacy. The significant variability in study findings probably relates to the variability in methods, particularly given the limited number of clinical trials.

The limited number of clinical trials investigating the effect of NAC on cisplatin-induced ototoxicity may reflect the difficulty in creating a compatible control group (Riga et al., 2013), particularly in human trials. The severity of cisplatin-induced hearing loss is influenced by numerous factors, many of which are unpredictable and obscure. Age, genetics, noise exposure and hearing acuity prior to cisplatin administration, individual and cumulative cisplatin dose, time intervals between cisplatin administrations, concomitant administration of other medical agents, general health, and renal function are some of the risk factors that are associated with cisplatin ototoxicity (Li et al., 2004; Mukherjea & Rybak, 2011; Roland, P.S., & Rutka, J.A.,
So, a controlled trial with a fully matched control group, which accounts for all of these variables, would require a large population. Since cisplatin-induced hearing loss is usually bilateral and symmetric, the treatment of one ear and the use of the other as control (“internal control method”) enables fully matched comparisons, even with limited sample sizes (Riga et al., 2013). Therefore, implementation of the internal control method in half (two human and two animal trials) of the reviewed studies was a justified research design. The remaining studies (one human and three animal trials) used control groups.

Another explanation for the relatively limited number of NAC clinical trials is the concerns about the potential of known otoprotective agents, including NAC, to react with cisplatin in systemic circulation, thereby provoking a probable compromise of anti-tumor efficacy (as cited by Yoo et al., 2013). It has been hypothesized that reduced anti-tumor effects from systemic drug interaction can be avoided by separating the routes of administration of otoprotective and chemotherapy treatments (Lorito et al., 2011; Riga et al., 2013; Yoo et al., 2013). Most of the studies included in the review of NAC chemoprotection have used two routes of administration (IT NAC versus IV or IP cisplatin) to minimize interactions between NAC and cisplatin. The adverse systemic effect of NAC on cisplatin efficacy, despite separate routes for NAC and cisplatin administration, was suspected in the study by Saliba et al. (2010). In the remaining studies that that separated the administration routes, no systemic drug interactions were reported, possibly suggesting that this separation achieved the goal of prevention of reduced anti-tumor effects resulting from undesirable drug interactions.

One more potential advantage of this route of administering NAC was the achievement of higher concentrations of protective drug in the inner ear. IT perfusion of the inner ear relies upon direct diffusion of the medication across the round window membrane and into the inner ear
fluids. This IT perfusion averts the blood-inner ear barrier and yields much higher concentrations of the chemoprotective agent in the inner ear as compared with the concentrations in the inner ear after systemic administration (Riga et al., 2013). Thus, IT perfusion allows direct treatment of the target organ for protection. Nonetheless, IT NAC did not yield significantly better otoprotection as compared with other routes of NAC administration. The successful application of IT, however, depends not only upon the protective properties of the chemoprotective agent, but also upon its diffusion characteristics across the round window membrane. Studies have shown that the round window membrane is highly permeable to drugs and chemicals with a molecular weight that is lower than 1000 Da (van den Berg et al., 2006). As previously mentioned, the molecular weight of NAC is 163.2 Da, which is well below the higher range of molecular weights of molecules that have been demonstrated to pass across the round window membrane. In all likelihood, therefore, NAC was readily transported across the round window membrane with IT perfusion.

The observed lack of significant otoprotective effect for IT NAC infusion may be related to difficulty in maintaining sufficiently high concentration in the middle-ear space to yield reliable diffusion into the inner ear. Since NAC solution is aqueous, the solution may have quickly drained out of the middle ear through the Eustachian tube shortly after middle-ear infiltration, thereby lowering the dose reaching the inner ear (Yoo et al., 2013). Another factor possibly accounting for the lack of significant otoprotective effect for IT NAC infusion may be the inflammatory response of the middle-ear mucosa induced by IT NAC in concentrations above 2%, as observed in all studies employing IT NAC concentrations in excess of 2%. Moreover, middle-ear inflammation in and of itself may augment cisplatin ototoxicity (Abi-Hachem, Zine, & Van De Water, 2010).
An additional limitation of the IT infusion technique used in the majority of the studies is that it is time-consuming. Although the IT injection takes only a few minutes, the study protocols required the participants to lie down (on the contralateral ear) for approximately 30 minutes to facilitate the diffusion of the otoprotective agent through the round window membrane (Nader et al., 2010; Riga et al., 2013; Saliba et al., 2010). Since cisplatin ototoxicity is bilateral, each ear needs to be treated, which would double the duration of the procedure.

The rationale for separation between the cisplatin and otoprotective agent administration routes and for the time interval between these administrations is based on minimizing drug interactions. Lorito et al. (2011) has speculated that the time interval between administrations of the otoprotective agent and cisplatin affects the outcome. Pre-administration of a thiol-containing antioxidant allows its accumulation in the inner ear before cisplatin reaches the target cells. Nonetheless, optimal NAC activity depends on the time window pre-administration; this is dictated by the NAC plasma half-life of 9 to 15 minutes, which results in rapid NAC clearance (as cited by Dickey et al., 2004). This fact seems to conflict with the finding (Dickey et al.) that an extended pre-treatment period is at least as effective as NAC administration immediately prior to cisplatin administration. Moreover, in the majority of studies for which at least partial NAC otoprotection occurred, the otoprotective agent was administered 30 minutes to 1 hour prior to cisplatin intervention, which is well beyond the NAC plasma half-life.

The optimal NAC dose may be critical for elicitation of its protective effect against cisplatin ototoxicity. At this time, no definitive conclusion regarding the optimal dose can be drawn due to significant dose variability among the studies relating to the type of subject (human versus animal), differences in the mode of otoprotective agent administration (e.g., IV versus IT), and variability in cumulative cisplatin dose; cumulative cisplatin dose needs to be considered
when interpreting study outcomes, since cisplatin ototoxicity is dose-dependent (Bertolini, Lassalle, Mercier, Raquin, Izzi, Corradini, & Hartmann, 2004a; Li et al., 2004; Reddel et al., 1982).

Many investigators have comprehensively detailed the adverse effects of cisplatin on the inner-ear structures, as manifested by the loss of auditory sensitivity caused by progressive destruction of the OHCs, inner hair cells (IHCs), and damage to supporting cells (Dickey et al., 2005; Feghali, Liu, & Van De Water, 2001a; Kopke et al., 1997). Histopathological analysis of the inner-ear structures performed in two of the reviewed studies revealed similar findings such as severe lesions of the OHCs and IHCs, with almost complete degeneration of stereocilia (Nader et al., 2010; Saliba et al., 2010).

Additionally, histopathological findings for the ears treated with NAC prior to cisplatin exposure have been presented in some of the reviewed studies. Saliba and colleagues (2010) reported less severe disturbance of the normal architecture of the organ of Corti in NAC-treated ears as compared with the control ears, possibly indicative of at least partial NAC otoprotective efficacy. But Nader and colleagues (2010) found no NAC preservative effect relating to the inner-ear structures. Conversely, cochlear analysis revealed severe disruption of the organ of Corti, with all cells affected. A significant difference in NAC concentration (4% versus 20%) employed by Saliba et al. and Nader et al. may account for these contradictory findings, with the 20% NAC concentration in the latter study resulting in a significant inflammatory reaction and diffuse osteitis, which could augment cisplatin ototoxicity (Abi-Hachem et al., 2010).
CONCLUSIONS

Presently, no ideal otoprotective agent exists in clinical use. Therefore, a safe and effective protective agent against cisplatin ototoxicity is greatly needed. This systematic review examined the efficacy of NAC prevention or amelioration of cisplatin ototoxicity. The findings of the reviewed studies show promise in preventing otologic damage with the use of the thiol compound, NAC (Choe et al., 2004; Dickey et al., 2004; Lorito et al., 2011; Riga et al., 2013; Saliba et al., 2010; Yildirim et al., 2010; Yoo et al., 2013). A shortcoming of NAC is that its protective effect, although significant, is only partial. Moreover, NAC administration is occasionally associated with adverse effects. Based on the currently available evidence, no recommendations for clinical practice can be made at this time.

Before definitive conclusions can be made about the NAC efficacy in patients treated with platinum-based chemotherapy, randomized controlled trials are needed to clarify NAC otoprotective efficacy as well as to determine the optimal NAC protocol (e.g., concentration, dosage, cumulative dose, timing and route of delivery). These randomized controlled trials should have homogenous study populations (with regard to, tumor diagnosis procedures and tumor severity) and have long-term follow-up. Also, the outcomes assessed should include ototoxicity, anti-tumor efficacy, otoprotective efficacy, adverse effects of otoprotective intervention, and quality of life.

If NAC proves to be safe and efficient at long-term follow-up, then it will have major implications for use in pediatric and adult populations who, after successful treatment for cancer, frequently are overwhelmed by the adverse effects of chemotherapy and their impact on quality of life (van den Berg et al., 2006). Not only would an effective otoprotective protocol eliminate
ototoxicity as one of the dose-limiting side effects of cisplatin therapy, but also it would improve quality of life for many patients.
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