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Original Research

Acetophenone protection against cisplatin-induced end-organ damage

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ABSTRACT

Cisplatin is a widely used and efficacious chemotherapeutic agent for treating solid tumors, yet it causes systemic end-organ damage that is often irreversible and detrimental to quality of life. This includes severe sensorineural hearing loss, hepatotoxicity, and renal injury. Based on the hard-soft acid-base theory, we recently developed two acetophenone-derived, enol-based compounds that directly interfere with the side effects of cisplatin. We investigated organ-specific and generalized toxicity in order to define dose-dependent responses in rodents injected with cisplatin with or without the protective compounds. All metrics that were used as indicators of toxicity showed retention of baseline or control measurements when animals were pre-treated with acetophenones prior to cisplatin administration, while animals injected with no protective compounds showed expected elevations in toxicity measurements or depressions in measurements of organ function. These data support the further investigation of novel acetophenone compounds for the prevention of cisplatin-induced end-organ toxicity.

Introduction

Cisplatin and other platinum-derived drugs are a mainstay of successful chemotherapeutic regimens for treating pediatric and adult solid cancers, including those of the bone and connective tissues, gonads, adrenals, and liver [1,2]. Cisplatin is administered intravenously and undergoes a molecular reorganization, known as aquation, in the cytoplasm of a cell, where one of its two chloride groups is replaced with a water molecule. This creates an increased affinity of the platinum core for nucleic acid nitrogen atoms and decreased affinity for sulfhydryl groups, which manifests into the DNA-disrupting mechanism of double strand breaks that leads to tumor cell death [3,4].

Despite the efficacy of platinum-based drugs, the off-target effects result in an over 60% incidence of often irreversible and debilitating organ damage such as ototoxicity, hepatotoxicity, and nephrotoxicity [3]. This occurs through excessive reactive oxygen species (ROS) generation that leads to metabolic oxidative stress, which disrupts mitochondrial activity and activates pro-apoptotic pathways [5]. Nearly 60% of children treated with cisplatin exhibit drug-induced sensorineural hearing loss [6]. Children under five years of age and children of the male sex are at greatest risk of suffering from ototoxicity, especially if the cumulative dose exceeds 400 mg/m^2 [6–8]. The severity of

Abbreviations: Gavinol, N-(4-acetyl-3-5-dihydroxyphenyl)-2-oxocytclop1entane-1-carboxamide; NAHA, 4-N-acetyl-2-6-dihydroxyacetaphenone.

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ototoxicity exhibits an inverse relationship with age at time of exposure, but any ototoxic impairment is irreversible and occurs on a dose-dependent basis [8]. Cisplatin also deposits in high concentrations in the kidneys, reaching toxic levels in a dose-dependent manner. An average of 30% of patients suffer from renal impairment due to cisplatin intravenous administration, which clinically manifests as increased creatinine levels, reduced glomerular filtration rates, and hypokalemia [9]. Hepatotoxicity is another major consequence of ROS production, oxidative stress, and mitochondrial impairment induced by cisplatin [10]. In rodent models, cisplatin has been shown to significantly increase lipid peroxidation (LPO) and protein oxidation and decrease activity of mitochondrial complex enzymes necessary for cellular respiration in the liver [11].

There is a scarcity of conclusive data on protective compounds that can temper the detrimental toxicity of cisplatin, presenting a complex problem for clinicians who attempt to balance the antineoplastic activity and off-target effects of chemotherapeutic agents [12]. The initial step of protecting against the toxic effects of cisplatin involves understanding the mechanism of injury on a molecular level. The hard and soft acids and bases (HSAB) model describes the nature of chemical interactions and bond formation through characterization of molecular species as "hard" or "soft." The hardness or softness of electrophiles and nucleophiles refers to their relative degree of electron density delocalization during covalent bond formation. Soft electrophiles have a distortable electron cloud when compared to hard electrophiles, which are characterized by a low degree of electron polarizability. Platinum contains both polarizable and non-polarizable electron densities that can form both hard-hard and soft-soft bonds, each with distinct biological consequences. Hard-hard bonds occur between cisplatin and DNA, which is the mechanism through which cisplatin exerts antineoplastic activity. Compounds with soft electrophilic character, like those found in platinum, react with soft nucleophiles, like sulfur residues, to form adducts of biological consequence. Some of these sulfur-based entities are cysteine residues of proteins like glutathione. Cisplatin also forms aggregates of intracellular protein adducts that may contribute to oxidative stress and resultant pro-apoptotic signaling [13]. The accumulation of these protein adducts leads to antioxidant depletion and initiates a mitochondrial injury cascade that involves the generation of ROS and metabolic stress, which is a common theme driving the debilitating downstream effects in end-organs due to ROS-induced interference with mitochondrial activity and activation of pro-apoptotic pathways [5,14–16]. Ototoxicity, nephrotoxicity, and hepatotoxicity are the downstream effects of the soft-soft interaction between platinum and various cellular entities [1,10,16–21].

We developed novel acetophenone compounds, 4-N-acetyl-2,6-dihydroxyacetaphenone (NAHA) and N-(4-acetyl-3,5-dihydroxyphenyl)-2-oxocytclopentane-1-carboxamide (Gavinol), that can act as nucleophilic surrogates for cisplatin's cytotoxic platinum-cysteine thiolate site (soft-soft) interactions that are at the molecular core of end-organ toxicity, such as in the cochlea, kidney, and liver. We have previously shown that the enol groups of NAHA and Gavinol ionize to form enolate in solution, creating a soft nucleophile that can interact with platinum without interfering with cisplatin's antineoplastic activity *in vitro,* which is mediated by hard-hard interactions [3]. Compared with sulfur-based N-Acetylcysteine (NAC) and sodium thiosulfate (STS), compounds previously investigated for their ability to mitigate the toxicity of cisplatin, our carbon-based acetophenone derivatives have longer half-lives (due to their intermediate level of lipophilicity) and can easily cross the cell membrane, making them readily bioavailable with low acute toxicity (LD50 >800mg/kg) [1,17–20]. Their smaller valence shells also promote reactive selectivity, making them less likely to participate in undesirable off-target reactions that may cause generation of ROS and subsequent cellular damage and toxicity.

In the present study, we tested the hypothesis that pre-treatment of rats with acetophenone compounds would protect against the toxic effects of cisplatin. Consistent with our hypothesis, we observed dose-dependent protective effects of NAHA and Gavinol on cochlear, renal, and hepatic injury associated with cisplatin administration. NAHA and Gavinol also preserved body weight and decreased oxidative stress when compared to vehicle control. These findings support the continued investigation of acetophenones to prevent toxic side effects of cisplatin chemotherapy.

Materials and methods

Animals

Male Sprague-Dawley juvenile rats were obtained from Charles River. They weighed between 150 and 200 grams. Rats were individually sheltered with access to food and water in a temperature-controlled room (22 °C ± 2 °C) with a 12 h light-dark cycle and a relative humidity of 55% ± 10%.

Chemical reagents

All reagents were purchased from Sigma-Aldrich (St. Louis, MO). NAHA and Gavinol were synthesized by The Chemical Synthesis and Biology Core Facility at Albert Einstein College of Medicine.

Treatment administration

Acetophenone compounds were dosed based on molar ratios relative to cisplatin but presented in μmol/kg. The lowest dose of NAHA and Gavinol was a 1:1 ratio of cisplatin to acetophenone, then it was increased to 1:2, 1:4, and 1:8. Acetophenone compounds or vehicle (PEG-400) were injected into rats 30 min prior to 30 μmol/kg cisplatin injection. Cisplatin was injected via slow intraperitoneal (IP) infusion. Acetophenones and vehicle injections were also injected IP.

Distortion product otoacoustic emissions (DPOAE) measurements to determine cochlear toxicity

Juvenile rats were anesthetized using a ketamine/xylazine cocktail and placed in a sound-dampening chamber on isothermal pads during DPOAE recordings. DPOAE measurements were performed on anesthetized rats using Tucker-Davis Technologies hardware (RZ6 Processor and W4 computer module) and software (BioSigRZ). For DPOAE testing, a single acoustic assembly containing an ER-10B microphone connected to two transducers (TDT, MF-1) was inserted into either of the rat's ear canals. An adapter was added on to the ER-10B microphone to create an acoustic seal between the microphone and tympanic membrane. Two primary tones were presented at fixed intensity levels of L1 = 65 dB SPL and L2 = 55 dB SPL at 11 f2 frequencies spanning 1-32 kHz and at unfixed f1 frequencies. The primary tone ratio used, f2/f1, was equal to 1.22, since this ratio has been previously shown to produce consistent and robust DPOAE at each tested frequency [22]. DPOAE signals were considered present if the response at a particular frequency was greater than the noise floor (Signal to Noise Ratio>0 dB). DPOAE measurements are used as an objective assessment of cochlear functioning [23].

Immediately before treatment, a DPOAE measurement was taken to create a composite control baseline (Day 0). Treatment groups included 30 μmol/kg cisplatin with or without NAHA or Gavinol at 4 concentrations: 239 μmol/kg, 119.5 μmol/kg, 59.8 μmol/kg or 32.3 μmol/kg. Initial DPOAE measurements were taken and all animals had similar hearing before treatment. At seven days post-treatment, another DPOAE measurement was taken and recorded to determine presence and severity of hearing deficits.

Body weight loss measurements as proxy for generalized toxicity [24]

Body weight measurements, as a proxy for generalized toxicity were obtained at Day 0 before treatment and at seven days post treatment.

Percent body weight loss was calculated for each subject. Each control, cisplatin-treated, and acetophenone-treated group contained five to thirteen rats.

Plasma measurements

Animals were decapitated in accordance with IACUC guidelines and whole blood was extracted from the neck of the animal with the use of sodium citrate as anticoagulant. Whole blood was passed through a 100-micrometer filter to exclude clotted blood and was centrifuged at 2000G for 15 min at room temperature. The supernatant/plasma was collected and used according to the assay protocol for each respective measurement.

Malondialdehyde (MDA) levels as a measurement of oxidative stress

The protocol for obtaining plasma was followed (as described above). The 1-methyl-2-phenylindole method was used to quantify MDA from whole blood samples [25]. Briefly, a reaction between 1-methyl-2--phenylindole, MDA, and 4-hydroxyalkenals produces a stable chromophore that is used to quantify the presence of MDA. This lipid peroxidation reaction allows for MDA yield to be measured at a 586 nm wavelength, at which the chromophore displays maximal absorbance. Each control, cisplatin-treated, and acetophenone-treated group contained three to eleven rats.

BUN and creatinine levels as measurements of kidney function

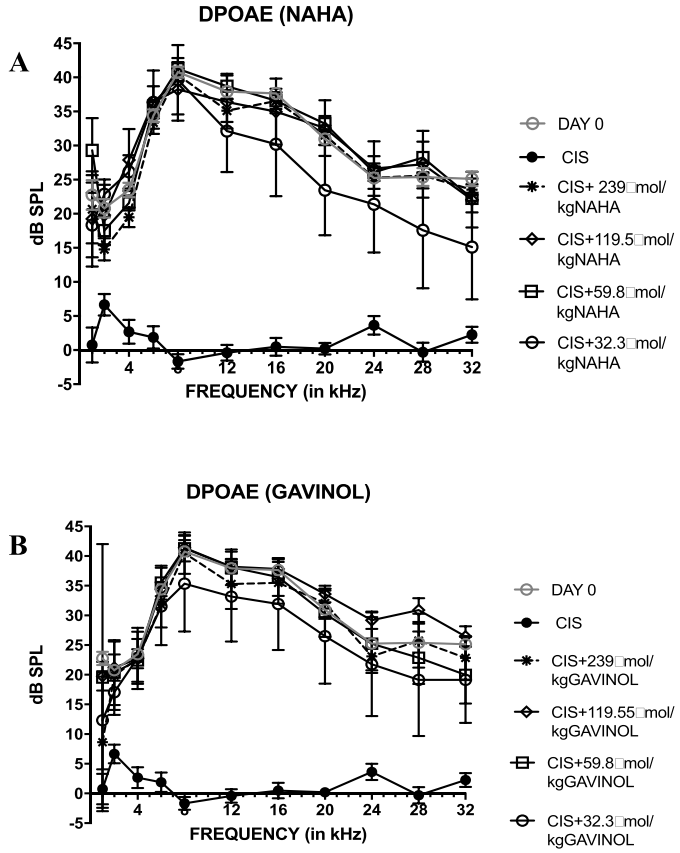
Creatinine measurements were obtained through liquid chromatography mass spectrometry via the University of Alabama-Birmingham chemistry core. The blood urea nitrogen (BUN) Colorimetric Detection Kit (ThermoFisher Scientific, catalog #EIABUN) protocol was followed [26]. Each control, cisplatin-treated, and acetophenone-treated group contained two to eighteen rats.

Alanine transaminase (ALT) measurements of liver functionality and hepatotoxicity

The protocol for obtaining plasma was followed (as described above). The Rat ALT ELISA Kit (abcam, catalog #ab234579) was used for ALT measurements [27]. Each control, cisplatin-treated, and acetophenone-treated group contained three to seven rats.

Statistical analyses

Statistical analyses were conducted in Prism 6.0 (Graphpad software; San Diego, CA). One-way ANOVAs with Tukey's multiple comparisons between groups for each measurement were conducted, with significance set at a probability of 0.05. P and F values are reported in the text adjoining each figure. The F-value is the ratio of inter-group variance and intra-group variance, and it provides more insight into the meaning of the p-value and the magnitude of difference between groups.



CONVERSION CHART		
	CISPLATIN	
uMol/Kg	Mg/Kg	
30	9	

CONVERSION CHART		
	GAVINOL	NAHA
uMol/Kg	Mg/Kg	Mg/Kg
239	50	66.28
119.5	25	33.14
59.8	12.5	16.59
32.3	6.75	8.97

Fig. 1. *DPOAE Measurements to Assess Cochlear Toxicity in Animals Treated with Cisplatin with or without Acetophenone Compounds***.** Sound pressure level (SPL in dB) of DPOAEs generated in cisplatin-only and cisplatin pre-treated with acetophenone compound groups are shown across the range of tone frequencies tested. Plots represent means; error bars represent ± 95% CI. Asterisks indicate the p-values obtained for each statistical comparison. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Different symbols represent different treatments and doses, as indicated in the legend. DPOAEs obtained from each animal before treatment (Day 0, gray curves) provided a baseline control measure of hearing at each frequency. Animals given cisplatin+vehicle showed near complete hearing loss after seven days (round black symbols).

Results

DPOAE analysis at each tested tone frequency provides a measure of the functionality of a specific region of the cochlea, thereby allowing for precise localization of hearing deficits [28]. Testing outer hair cell functioning seven days post-exposure revealed auditory protection by NAHA and Gavinol across all tested tone frequencies, with DPOAEs being significantly larger than the near-zero sound pressure level (SPL) of DPOAEs observed in animals given only cisplatin (Fig. 1A & B). At the lowest dose of acetophenone compounds administered (32.3 μmol/kg), protection against cisplatin ototoxicity was provided at a hearing frequency of 8 kHz, as demonstrated by the DPOAEs of 39.7 ± 2.4 (NAHA) and 35.4 ± 3.9 dB SPL (Gavinol) elicited in the acetophenone-treated rats (Fig. 1A & B). Analysis at 12 kHz revealed overlapping magnitudes of DPOAEs in untreated and treated rats with cisplatin co-administration with 59.8 μmol/kg NAHA, and cisplatin co-administration with 59.8 μmol/kg Gavinol (37.9 ± 0.3 dB, 38.7 ± 1.8 dB, and 38.1 ± 1.4 dB, respectively). There was a statistically significant difference between DPOAE SPLs at baseline and those in subjects co-treated with cisplatin and 32.3 μmol/kg of NAHA, indicating that the lowest dose of acetophenone protectant did not offer as much protection as other doses (Fig. 1A). The same pattern occurred with animals treated with cisplatin and 32.3 μmol/kg of Gavinol at 1 kHz, 16 kHz, 28 kHz, and 32 kHz (Fig. 1B). Increasing the dose of acetophenone compounds beyond 59.8 μmol/kg provided no additional significant protection, suggesting that dosage does not have to approach higher concentrations to be effective. For NAHA-treated groups, $F(50, 3539) = 8.5$. For Gavinol-treated groups, $F(50, 3637) = 7.5$. Single agent NAHA did not affect DPOAE measurements (See Supplemental Material Fig. S1).

We further found that acetophenones protected against cisplatin-induced generalized toxicity as assessed via body weight. Animals receiving cisplatin at a dose of 30 μmol/kg without co-administration of acetophenone compounds showed a 28.4 ± 0.6% reduction in body weight seven days after treatment. The lowest NAHA and Gavinol doses reduced the magnitude of weight loss to 18.6 ± 2.8% (Fig. 2A) and 21.3 ± 1.1% (Fig. 2B), respectively. Animals receiving 239 μmol/kg of acetophenone compounds retained a significant amount of body weight, losing only 14.9 ± 2.7% (NAHA, Fig. 2A) and 12.7 ± 4.5% (Gavinol, Fig. 2B). These data also reveal a trend toward greater protection at higher doses. By day 9 after acetophenone treatment, animals cease losing body weight and start to recover the mass that was lost in all NAHA- and Gavinol-treated groups (data not shown). For NAHA-treated groups, $F(4, 42) = 5.3$. For Gavinol-treated groups, $F(4, 50) = 6.0$. NAHA given without cisplatin had no significant effect on body weight, indicating that acetophenones do not promote generalized toxicity (see Supplemental Material Fig. S2).

Malondialdehyde (MDA) serum concentration is an indirect measure of oxidative stress. MDA is produced when ROS degrade poly-unsaturated lipids through lipid peroxidation. MDA levels were measured seven days after treatment with cisplatin+vehicle or cisplatin co-administered with each of the two acetophenone compounds. Rats receiving 30 μmol/kg cisplatin without protective compounds produced 6.0 ± 2.295 μmol/mL of MDA, whereas rats receiving vehicle alone produced 1.9 ± 0.3 μmol/mL of MDA (Fig. 3). All four doses of NAHA and Gavinol significantly protected against an increase in MDA from cisplatin. For NAHA-treated groups, $F(5, 27) = 8.0$. For Gavinol-treated groups, $F(5, 26) = 8.0$.There were no significant differences between rats treated with vehicle alone and those treated with acetophenone compounds.

In addition to hearing loss, cisplatin induces nephrotoxicity, reflected by increases in serum blood urea nitrogen (BUN) and creatinine levels. BUN was used as a measurement of kidney function, since urea is filtered from the blood by the kidneys as a byproduct of metabolism. Similarly, serum creatinine is a metabolic byproduct of the kidneys, but it is filtered at a steady rate and used to normalize BUN values. It is a clinically relevant marker of the kidneys' ability to filter waste from the blood. Administration of 30 μmol/kg cisplatin resulted in significant increases in BUN and creatinine to 14.0 ± 1.4 mg/dL and 1.5 ± 0.13 μg/mL, respectively, compared to vehicle-treated animals (baseline BUN 0.2 ± 0.2 mg/dL and creatinine 0.06 ± 0.01 μg/mL), confirming kidney dysfunction as a result of direct nephrotoxicity from the platinum agent [12]. NAHA and Gavinol provided significant protection that resulted in smaller increases of BUN and creatinine at all four doses as compared with increased observed in the cisplatin-alone group. Administration of NAHA and Gavinol reduced BUN levels to a range of 4.0 ± 1.7 mg/dL to 5.5 ± 2.8 mg/dL and 4.1 ± 1.7 mg/dL to 6.9 ± 2.3 mg/dL, respectively (Fig. 4A & B). Administration of NAHA and Gavinol reduced creatinine levels to a range of 0.4 ± 0.2 μg/mL to 0.6 ± 0.2 μg/mL and 0.5 ± 0.2 μg/mL to 0.7 ± 0.2 μg/mL, respectively (Fig. 4C & D). Differences in protection between doses were not statistically significant. More specifically, for groups co-treated with NAHA, BUN data revealed $F(3, 16)$

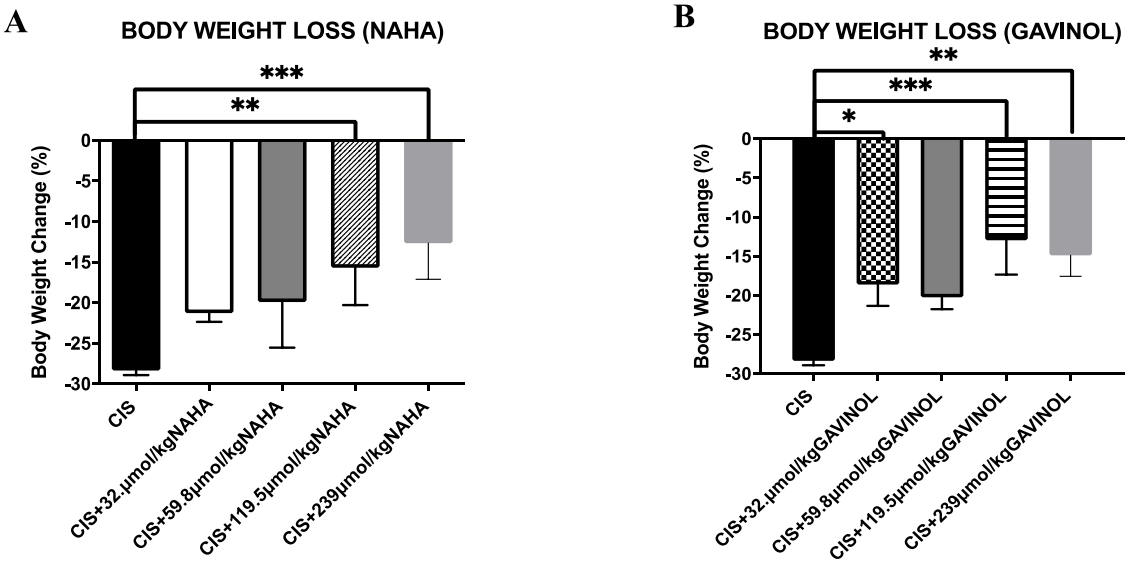


Fig. 2. *Body Weight Loss Measurements to Assess Generalized Toxicity*. The rodents' body weight was measured immediately before treatment and seven days after treatment. Plots represent means; error bars represent ± SEM. Asterisks indicate the p-values obtained for each statistical comparison. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

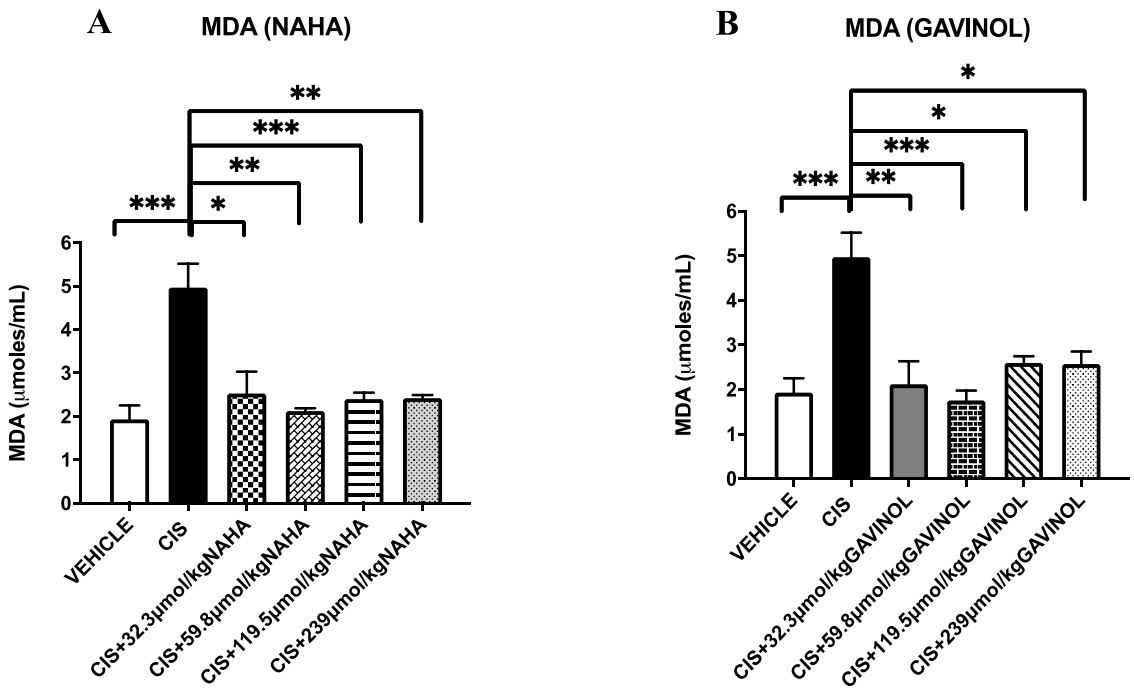


Fig. 3. *Malondialdehyde (MDA) Measurements to Assess Oxidative Stress Magnitude*. Cisplatin without protectant induces a significant amount of MDA production. Plots represent means; error bars represent ± SEM. Asterisks indicate p-values associated with each post-hoc test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

= 0.2 with p = 0.9 and creatinine data revealed F(3, 15) = 0.1 with a p = 1.0. For groups co-treated with Gavinol, BUN data revealed F(3, 18) = 0.2 with p = 0.9 and creatinine data revealed F(3, 17) = 0.2 with p = 0.9.

ALT is a key liver-specific enzyme in gluconeogenesis and amino acid degradation. Elevated levels of ALT in the plasma reflect hepatocellular injury and cell death, with higher levels indicating a greater level of liver injury [15]. ALT was significantly elevated to 2550 ± 358 ng/mL when 30 μmol/kg of cisplatin was administered. In contrast, rats receiving vehicle alone displayed ALT levels of 868 ± 52 ng/mL of ALT (Fig. 5A and B). ALT remained at near vehicle levels when cisplatin was co-administered with 32.3 μmol/kg of NAHA and Gavinol (852 ± 253 ng/mL and 834 ± 173 ng/mL, respectively) and with all doses of NAHA and Gavinol (e.g., 1028 ± 2045 ng/mL and 852 ± 225 ng/mL with 239 μmol/kg NAHA and Gavinol, respectively) (Fig. 5A & B). All doses of acetophenone compounds protected rats against liver injury, as indicated by significant reductions in ALT relative to values in the cisplatin alone condition. For the NAHA-treated groups, F(5, 27) = 8.1. For the Gavinol-treated groups, F(5, 25) = 6.5. All doses provided roughly equal protection, with no significant differences observed between doses.

Discussion

The present study demonstrates that pre-treatment with the acetophenone compounds, NAHA and Gavinol, provides powerful protection against end-organ damage caused by cisplatin administration, as assessed by measures of DPOAEs, body weight retention, MDA levels, BUN and creatinine levels, and ALT serum concentration. The significant prevalence of cisplatin-induced end-organ damage and the simultaneous efficacy of cisplatin as an antineoplastic drug, used in approximately 40% of chemotherapy treatments, highlight the compelling need for an agent that protects against cisplatin toxicity. The scavenging molecular mechanism of NAHA and Gavinol is well established, but the clinical protective effects against cisplatin toxicity has yet to be clearly demonstrated [3,17–20]. To our knowledge, the present study is the first to provide direct evidence for the prevention or reduction of cisplatin-induced end-organ damage in a rodent model with the use of these acetophenone compounds.

The first measure of prevention of end-organ damage was obtained through DPOAE recordings. DPOAE magnitude is a sensitive and rapid measure of ototoxic alterations in cisplatin-treated animals and humans [29]. The presence of normal DPOAE levels supports the integrity of sound amplification mechanisms in the cochlea, while the absence of or decreased DPOAEs indicates dysfunction of this amplification system [14]. Clinically, cisplatin-induced cochlear toxicity manifests as initial damage to high-frequency hearing. With increasing cumulative doses, damage progresses to regions responsible for lower-frequency hearing [30–32]. The retention of near-normal levels of DPOAEs observed in acetophenone-treated animals across all dosages supports the remarkable efficacy of acetophenone compounds to prevent cisplatin-induced ototoxicity [33]. These findings are clinically significant, given that hearing loss associated with cisplatin administration negatively impacts speech perception and language development [34,35]. Moreover, hearing-impaired children are more likely to develop attention deficits, behavioral problems, and associated difficulties in social and academic settings [36].

Weight loss is a well-established marker for general toxicity and cachexia proves to be a limitation in chemotherapeutic treatment of cancers using cisplatin [24,37]. Cisplatin suppresses lipogenesis and subsequent adipose deposition, contributing to overall reduced body weight [38]. Administration of acetophenone led to body weight retention in a dose-dependent manner, suggesting that use of these compounds may protect patients from poor survival outcomes associated with cisplatin-induced cachexia or anorexia [37].

One of the causes of oxidative stress, which is the mechanism underlying cisplatin-induced end-organ damage, is LPO that produces MDA as a byproduct of this process. MDA is a thoroughly investigated indicator of cell membrane damage via ROS production. Directly measuring ROS levels proves to be ineffective and inefficient given their limited lifespan and unstable reactivity. Therefore, quantifying the cellular damage via measuring ROS-mediated lipid peroxidation is a reliable alternative [39]. Not only does MDA accumulation occur as a result of tumorigenesis in cancer pathology, but also as a consequence of cisplatin administration once the drug enters and acts upon cell

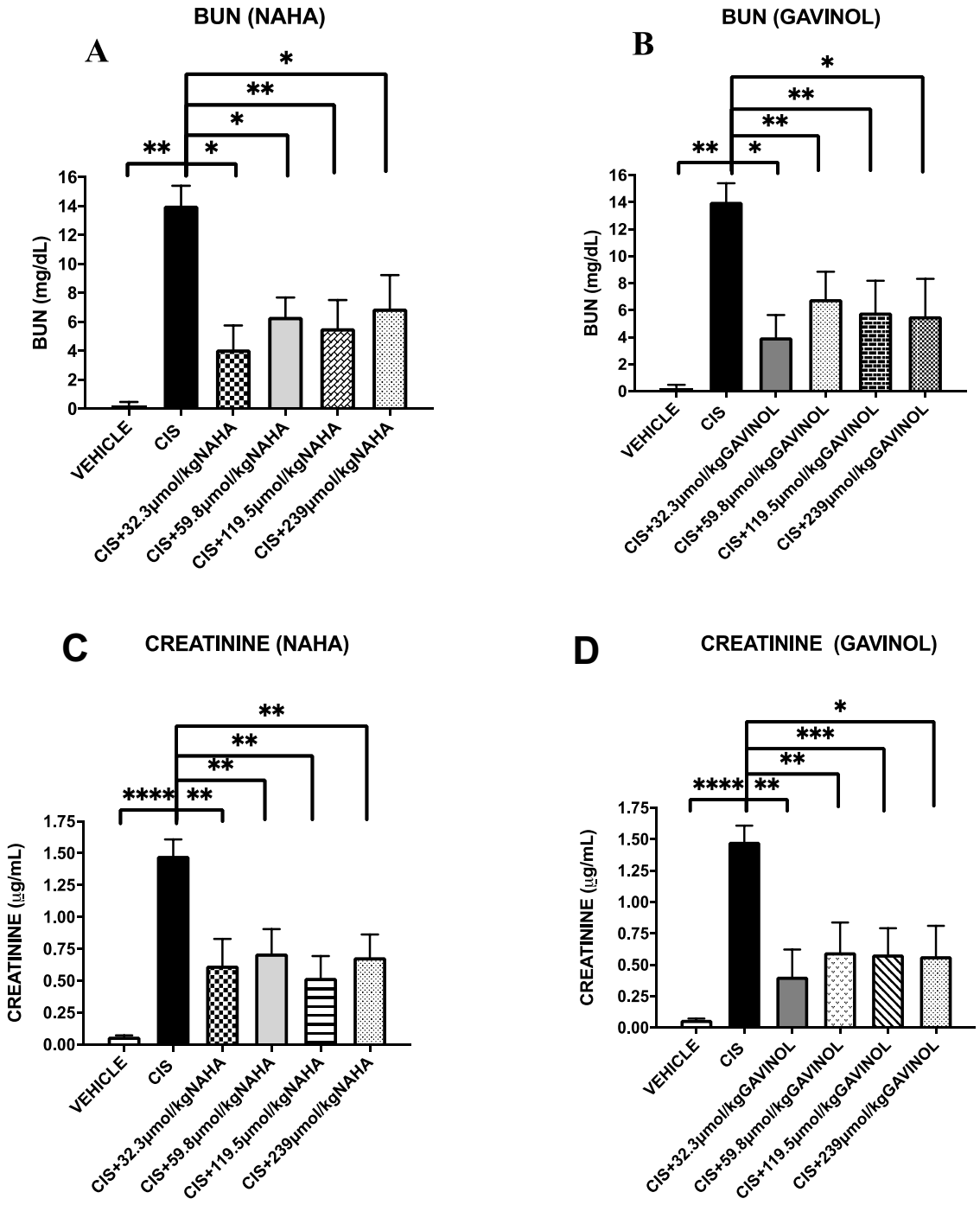


Fig. 4. *Concomitant BUN and Creatinine Measurements to Assess Kidney Function*. Animals treated with cisplatin and an acetophenone protectant show significantly reduced increases in levels of BUN (A and B) and creatinine (C and D). Plots represent means; error bars represent ± SEM. Asterisks indicate the p-values obtained for each statistical comparison. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

A. Measurements in animals treated with NAHA show significant reductions in BUN production across all doses. $F(5, 34) = 5.4$.

B. Measurements in animals treated with Gavinol show significant reductions in BUN production across all doses.

membranes [39,40]. We found that co-administration of acetophenones significantly reduced the magnitude of MDA produced by cisplatin. These results demonstrate an overall decrease in oxidative stress, suggesting acetophenones can markedly attenuate the mechanism through which end-organ damage occurs.

Another common consequence of cisplatin use is nephrotoxicity, which is evidenced by simultaneous spiking of BUN and creatinine. Cisplatin's nephrotoxic effects may manifest as acute kidney injury (AKI), hypomagnesemia, Fanconi-like syndrome, hypocalcemia, and distal renal tubular acidosis [14,9]. In human subjects, BUN and creatinine become elevated as a result of cisplatin administration, establishing the foundation for these disturbances as reliable markers of acute nephrotoxicity [13]. NAHA and Gavinol prevent the elevation of both BUN and creatinine, indicating partial protection against severe kidney injury that is seen in animals that received cisplatin alone. Moreover, no statistically significant difference between control groups and acetophenone-treated groups were observed. Beyond the doses tested in the present study, other doses of acetophenone compounds may produce

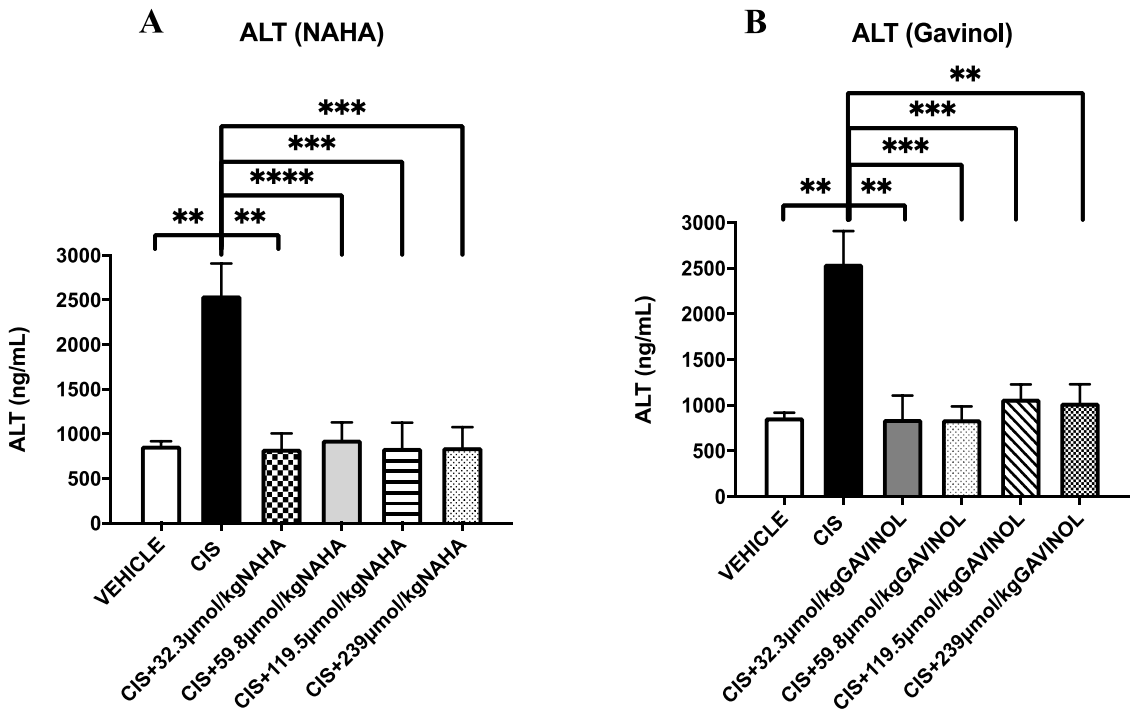


Fig. 5. *Alanine Aminotransferase (ALT) Measurements to Assess Liver Functionality and Severity of Hepatotoxicity*. Cisplatin-treated animals that are given NAHA (A) or Gavinol (B) show significantly decreased ALT relative to animals treated only with cisplatin. Plots represent means; error bars represent ± SEM. Asterisks indicate the p-values obtained for each statistical comparison. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

an even greater magnitude of protection. Moreover, different time points in measurements of serum BUN and creatinine levels that extend beyond seven days post-treatment may uncover various magnitudes of protection.

Hepatotoxicity is yet another downstream consequence of oxidative stress induced by cisplatin. Hepatocellular injury manifests as increased serum ALT levels, as shown by previous research in which rodents treated with cisplatin showed significant elevations in ALT levels [15,11]. We found that cisplatin treatment leads to increased serum ALT, with significant protection observed when acetophenone compounds are administered 30 min to one hour prior to cisplatin administration [41]. Taken together, our findings provide novel evidence for acetophenone protection against nephrotoxicity and hepatotoxicity, given that proof of systemic end-organ rescue after cisplatin administration has not been established previously [42,12,41].

One important limitation of our work is that we did not use tumor-bearing animals, thus hindering our ability to determine whether cisplatin retains its effectiveness as a chemotherapeutic agent when co-administered with acetophenones. In addition, in the future it will be important to distinguish whether acetophenone compounds rescue the tissue after damage has occurred or if they only prevent toxicity from occurring in the first place. Furthermore, since our study focused on only two time points, baseline and seven days after treatment, it was not possible to evaluate the temporal dynamics of cisplatin-induced toxicity and its amelioration by acetophenones occurring within this time frame. The cisplatin dose evaluated in the present study does not resemble the clinical regimen of cisplatin administration, which is a multi-day high-dose course separated by recovery periods. In order to determine the appropriate protective acetophenone dose, follow-up experiments with clinically similar cisplatin doses will be conducted. Moreover, it would be important to investigate whether and how various routes of cisplatin administration affect end-organ toxicity outcomes. Finally, evaluation of peripheral neurotoxicity is ongoing. Up to 80% of patients can experience nerve damage after treatment with cisplatin [43]. Platinum accumulation in dorsal root ganglion (DRG) sensory neurons and the formation of platinum-DNA adducts results in cisplatin-induced mitochondrial DNA damage, increased intracellular ROS, and channelopathies [44,45]. *In-vitro* studies reveal that cisplatin induces loss of significant viability in DRG cells and hepatocytes [3]. Although our previous *in vitro* work established the superiority of acetophenone compounds in cisplatin-induced cytotoxicity prevention in rat DRG cells and hepatocyte cells relative to the protective effects of sulfur-compounds [3], we do not yet have data related to the efficacy of protective compounds that may reverse or prevent such deleterious downstream consequences of cisplatin-based chemotherapeutic regimens *in-vivo* [12,45]. Assessing whether neurotoxicity can also be prevented *in vivo* is a vital next step.

Cancers like medulloblastoma, osteosarcoma, hepatoblastoma, and neuroblastoma are aggressive malignancies of childhood with dismal survival rates for patients with metastatic disease [28,46,47]. For survivors, the therapeutic regimens that children endure have long-lasting impacts on their quality of life as they enter into adolescence and adulthood. In particular, platinum-based treatments have a well-characterized toxic profile. Establishing the molecular mechanism of cytotoxicity through the HSAB model propelled us to develop novel agents that attenuate the pernicious downstream effects of cisplatin. Our studies on rodent cochlea, kidney, liver, and oxidative damage measures provide an important foundation for further exploration of the ability of acetophenone compounds to protect patients from cisplatin-induced end-organ damage. Our future studies will include animal models with tumors to ensure there is no interference with anti-cancer activity as well as histological analyses of end-organ damage and acetophenone protection. Crucially, the present findings demonstrate that NAHA and Gavinol have the potential to ease the burden of clinical decision-making by eliminating the need for patients and their families to choose between impaired quality of life or cisplatin-based intensive chemotherapy.

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CRediT authorship contribution statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2022.101595.

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