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To cite this article: David M. Lehmann & Wanda C. Williams (2019): Cross-reactivity between halogenated platinum salts in an immediate-type respiratory hypersensitivity model, *Inhalation Toxicology*, DOI: [10.1080/08958378.2018.1554015](https://doi.org/10.1080/08958378.2018.1554015)

To link to this article: <https://doi.org/10.1080/08958378.2018.1554015>



Published online: 18 Jan 2019.



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RESEARCH ARTICLE



Cross-reactivity between halogenated platinum salts in an immediate-type respiratory hypersensitivity model

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ABSTRACT

Halogenated platinum salts can trigger the development of occupational asthma. Until recently, laboratory research into the development and manifestation of platinum hypersensitivity responses were hindered by the lack of an animal model suitable for assessing the functional consequences of allergic sensitization. We employed a newly developed mouse model to assess the potential allergenicity of ammonium tetrachloroplatinate (ATCP), compare the relative potency of ATCP and another platinum salt, ammonium hexachloroplatinate (AHCP) and assess potential cross-reactivity. Mice were topically sensitized with ATCP before being challenged by intratracheal aspiration (IA) with ATCP. Ventilatory responses were assessed using whole-body plethysmography (WBP). An immediate response (IR) was observed in ATCP-sensitized and challenged mice. Two days later, responsiveness to the nonspecific stimuli methacholine (Mch) was detected in ATCP-sensitized mice using WBP. Bronchoalveolar lavage fluid collected from sensitized mice contained an average of 3.3% eosinophils compared to less than 0.5% in non-sensitized mice ($p < .05$). Serum harvested from sensitized mice also contained increased total serum immunoglobulin E ($p < .05$). These data are the first to demonstrate that topical exposure to ATCP is sufficient to develop immediate type hypersensitivity and that a single intra-airway challenge is capable of triggering pulmonary responses. To investigate potential cross-reactivity, mice were sensitized to AHCP and, challenged by a single IA with a second platinum compound, ATCP. Compared to non-sensitized mice challenged with ATCP, these mice exhibited an IR, responsiveness to Mch, and eosinophilic infiltration in the lungs similar to that achieved with AHCP challenge, thus demonstrating cross-reactivity.

ARTICLE HISTORY

Received 27 August 2018
Revised 23 October 2018
Accepted 20 November 2018

KEYWORDS

Platinum; ammonium hexachloroplatinate; ammonium tetrachloroplatinate; occupational asthma; respiratory hypersensitivity; cross-reactivity; whole-body plethysmography; pulmonary hyperresponsiveness

Introduction

Asthma is a condition associated with narrowing of the airways leading to bouts of chest tightness, wheezing, coughing and shortness of breath. Occupational asthma is the most common occupational lung disease, accounting for up to 15% of asthma cases in the United States (ALA, 2008; Balmes et al., 2003; Blanc & Toren, 1999). Asthmatic reactions can be triggered by exposure to high concentrations of irritants (e.g. hydrochloric acid, sulfur dioxide, ammonia, smoke) or through the development of a type I hypersensitivity response (e.g. isocyanates, metals). Unlike irritant-induced asthmatic reactions which occur immediately after first exposure, type I hypersensitivity reactions occur after repeated work-related exposure to an allergy-inducing substance resulting in activation of an adaptive immune system response. Continued exposure to occupational asthmagens can intensify symptoms and lead to the development of life-long asthma. Occupational asthma has the potential to not only adversely impact worker productivity, but to also decrease the quality of life for affected individuals. Consequently, identification and characterization of occupational asthmagens is paramount.

Halogenated platinum salts are a well-known occupational health hazard associated with type I hypersensitivity, accompanied by conjunctivitis, rhinorrhea, sneezing, cough and urticaria (reviewed in IPCS, 2012; WHO, 2000). Immediate type respiratory hypersensitivity reactions resulting from exposure to platinum salts have been reported in up to 50% of exposed workers during the manufacturing of catalysts (Hughes, 1980; Roberts, 1951). In a 5-year prospective cohort study, 11% of workers exposed to 52.9 ± 19.7 ng soluble Pt/m³ in a catalyst production plant developed positive skin prick tests (Merget et al., 2000). Importantly, symptoms may worsen in sensitized individuals with continued exposure (Levene, 1971) and, in some cases, removing the affected individual from the platinum-containing environment was not sufficient to alleviate the symptoms of platinum hypersensitivity (Merget et al., 1994).

Cross-reactivity between antigens occurs when an antibody directed against one antigen binds to a different antigen (Chadwick, 2007). A few human studies of cross-reactivity among platinum group metals (PGMs) and between platinum compounds has been reported (Cristaudo et al., 2005; Hartmann & Lipp, 2003; Markman et al., 2003;

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Murdoch & Pepys, 1985, 1986, 1987; Santucci et al., 2000). Cross-reactivity among platinum-containing anticancer drugs has also been reported and represents a serious concern for medical practitioners because patients are at risk of potentially hypersensitivity reactions to related therapeutic agents (Hartmann & Lipp, 2003). In the only published study investigating cross-reactivity between chloroplatinates, Murdoch & Pepys (1985) investigated cross-reactivity of IgE antibodies produced in response to sensitization to platinum-ovalbumin conjugate with various platinum compounds and found that female Hooded Lister rats developed antibodies to intraperitoneally injected platinum-OVA conjugate and that this response sensitized the rats to platinum-BSA as well as free ammonium tetrachloroplatinate (ATCP) and ammonium hexachloroplatinate (AHCP). Whether, or not cross-sensitivity produced changes in lung physiology was not investigated in this study or any other. The potential for cross-reactivity among platinum compounds has important implications for human health risk assessment and warrants further investigation.

Limited animal-based research has been conducted to characterize the effects of exposure to halogenated platinum salts. The sensitizing effects of inhaled halogenated platinum salts were investigated in two published studies (Biagini et al., 1983, 1986). Cynomolgus monkeys exposed to aerosolized ammonium hexachloroplatinate experienced increased bronchial hyperreactivity to both platinum and nonspecific stimuli (i.e. methacholine, Mch) (Biagini et al., 1983). Topical treatment of mice with various halogenated platinum salts results in immune responses consistent with sensitization (Ban et al., 2010; Basketter et al., 1999; Dearman et al., 1998, 2013; Schuppe et al., 1992, 1997, 1997; Williams et al., 2014). However, it was not known if dermal sensitization to halogenated platinum salts was sufficient to trigger changes in lung function following respiratory challenge with platinum. Recently, we developed the first murine model of halogenated platinum salt sensitization with the capacity to assess respiratory responses (Williams et al., 2015). Using this model, we showed for the first time that mice dermally sensitized with AHCP (1) exhibit an immediate response (IR) and experience eosinophilic infiltration of the lung following delivery of AHCP to the respiratory tract, (2) are responsive to the nonspecific stimuli Mch and (3) have significantly elevated total immunoglobulin E (IgE) in their serum (Williams et al., 2015). Here, we use this model to assess the development of immediate type hypersensitivity to another halogenated platinum salt, ATCP, enabling us to compare the relative potency and potential cross-reactivity of these two platinum compounds.

Materials and methods

Animals

Female BALB/c mice (8–9 weeks old at study initiation) were purchased from Charles River Laboratories (Wilmington, MA). Mice were housed in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-approved facility (21.5 °C ± 1.5 °C,

relative humidity of 55%±5%, and a 12 h light/dark cycle). Mice were group-housed based in polycarbonate cages with hardwood chip bedding (NEPCO, Warrensburg, NY) and were provided a balanced diet mouse chow (5POO Prolab RMH3000, PMI Nutrition International, Richmond, IN) and water *ad libitum*. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of NHEERL, US EPA.

Chemicals

Test substances used for this study, including AHCP (99.995% purity), ATCP (99% purity) and acetyl-β-Mch chloride, were purchased from Sigma Aldrich (St. Louis, MO). AHCP and ATCP were prepared in dimethyl sulfoxide (DMSO; Biomatik, Wilmington, DE) for dermal dosing or pyrogen-free 0.9% sodium chloride, injection, USP (Hospira, Inc, Lake Forest, IL) for delivery to the airways.

Sensitization and challenge protocol

Mice were sensitized and challenged as previously reported (Williams et al., 2015). Briefly, mice were dosed (sensitized) topically with either 1% ATCP or 1% AHCP (in DMSO) on the shaved back (100 µL) on experimental days 0, 5 and 15 and on the ears (25 µL/ear) on experimental days 10, 11 and 12 (Figure 1). Control mice received vehicle only. Mice were challenged with ATCP (10, 31 or 100 µg prepared in saline) or AHCP (100 µg prepared in saline) through the airways by intratracheal aspiration (IA) on experimental day 24. This procedure has been shown to deliver material directly to the lungs (Foster et al., 2001). As described previously, anesthetized mice (3.5% isoflurane in oxygen) were suspended by their frontal incisors, and, using forceps, the tongue was gently pulled to one side to allow dosing to the oropharynx. After test article placement, the nose was briefly occluded to encourage the mouse to inhale through its mouth (Williams et al., 2015). The 100 µg dose of AHCP was chosen based on our previous study (Williams et al., 2015). As described previously (Williams et al., 2015), ventilatory responses to ATCP and AHCP were measured in unrestrained, unanesthetized mice using whole-body plethysmography (WBP; DSI, St. Paul, MN) immediately after IA delivery. Enhanced pause (Penh), a quantifiable indicator of pulmonary hyperresponsiveness (Farraj et al., 2007; Hamelmann et al., 1997), was calculated using Buxco BioSystem XA software (DSI, St. Paul, MN). On experimental day 26, respiratory responses to three escalating doses of aerosolized Mch were also measured using WBP. For both pulmonary responses, average values were collected for 10 min prior to IA delivery of platinum compound. Immediately following IA delivery of the platinum compound, measurements were collected for 60 min and were used to determine Penh. Average Penh values were calculated for each animal over the entire measurement period (50 measurements for the 10-minute baseline period and 300 measurements for the 60-minute post-instillation period). Average values for each animal were used

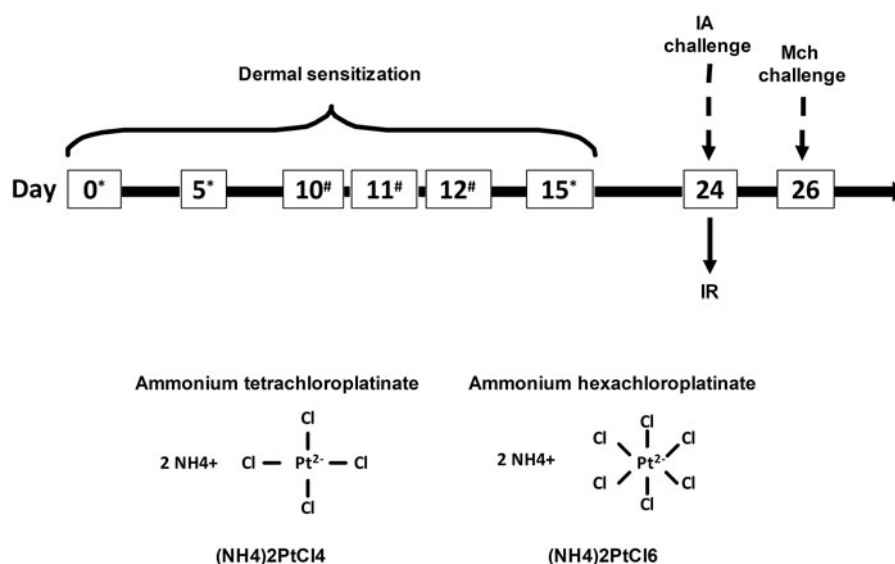


Figure 1. Exposure regimen. BALB/c mice were dosed with test article/vehicle topically on the shaved back on experimental days 0, 5 and 15 (*). Test article/vehicle was applied to the dorsum of both ears on experimental days 10, 11 and 12 (#). Mice were challenged by intratracheal aspiration (IA) with AHCP on experimental days 24. Immediate responses (IR) were measured by whole-body plethysmography. On experimental days 26, pulmonary responses to aerosolized methacholine (Mch) were measured by WBP.

subsequently to calculate the group average Penh values (expressed $\pm$ standard error of the mean) (Figure 1).

Blood collection and euthanasia

At study termination (i.e. day 26), mice were administered Euthasol (Virbac AH, Inc, Fort Worth, TX) 1 h after Mch challenge and then exsanguinated by cardiac puncture. Blood was collected in serum collection tubes (BD Falcon, San Jose, CA) and processed to serum according to the manufacturer's instructions prior to being stored at -80°C .

Lymph node cell collection

The intrapulmonary/tracheobronchial (mediastinal) and auricular lymph nodes (ALN) were collected as described previously (Williams et al., 2015). Briefly, excised lymph nodes were placed in room temperature RPMI 1640 tissue culture media containing 25 mM HEPES, 2.05 mM L-glutamine (Invitrogen, Grand Island, NY), 10% fetal bovine serum (Hyclone, Logan, UT) and 2% penicillin/streptomycin (Cellgro, Manassas, VA) before being mechanically disaggregated and passed through a 100 μm Celltrics filter (Partec, Munster, Germany). Lymph node cells were pelleted by centrifugation ($300\times g$ for 7 min at room temperature) and resuspended in 1 mL complete RPMI 1640. Using a Coulter Counter (Beckman Coulter, Brea, CA), lymph node cells were counted. Viability was determined by trypan blue dye exclusion.

Bronchoalveolar lavage fluid (BALF) recovery and processing

As previously described (Williams et al., 2015), BALF was collected from mice immediately after euthanasia. Briefly, after cannulating the trachea and clamping off the left lung,

the right lung was washed three times with 0.6 mL room temperature Ca^{2+} , Mg^{2+} and phenol red-free HBSS (Life Technologies, Bethesda, MD). Approximately, 85% of the instilled volume was recovered in all treatment groups. The second two washes were pooled. All washes were centrifuged ($360\times g$ for 15 min at 4°C) to pellet BALF cells. The supernatant from the first wash was used for lactate dehydrogenase (LDH) and total protein measurements. The cell pellets from all three washes were pooled and resuspended in 1 mL Ca^{2+} , Mg^{2+} and phenol red-free HBSS and used for cell counts differentials.

BALF cell counts and differentials

Cells (200 μL of suspension) were cytocentrifuged (Shandon Southern Instruments, Sewickley, PA) onto duplicate slides (200 rpm for 10 min). After drying, the slides were stained with Wright Giemsa Stain Pack (Fisher Scientific, Suwanee, GA) using a Hema-tek 2000 (Miles Inc., Elkhart, IN). Differential cell categorization (macrophages, neutrophils, lymphocytes and eosinophils) was determined by counting 200 total cells per slide.

Total serum IgE detection

Total serum IgE was determined using a commercial ELISA-based colorimetric assay kit according to the manufacturer's instructions (BD Pharmingen, San Diego, CA). Briefly, 96-well flat-bottom Costar microtiter plates (Corning Life Sciences, Tewksbury, MA) were coated with anti-mouse IgE antibody (BD Pharmingen, San Diego, CA) and incubated overnight at 4°C . After blocking the plates with 1% bovine serum albumin (Sigma Aldrich, St. Louis, MO), serum samples (1:10 dilution non-sensitized mice, 1:60 and 1:90 sensitized mice) were added to the wells and allowed to incubate at room temperature for 1 h. After

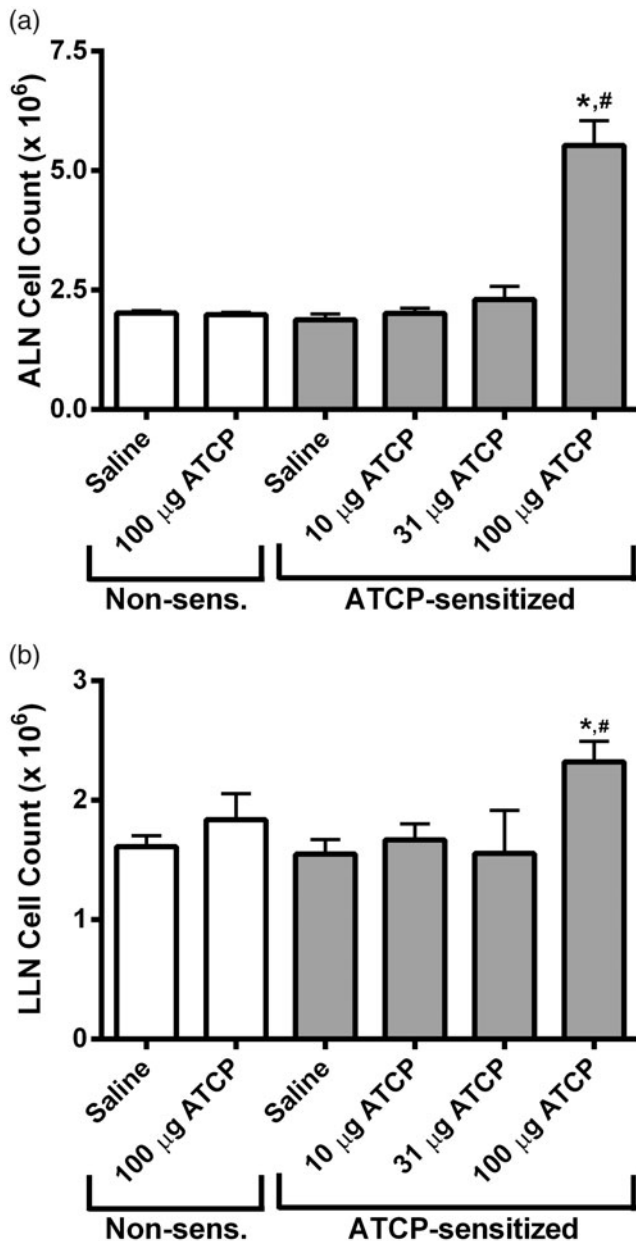


Figure 2. Assessment of ATPC treatment on lymph node cell proliferation by direct cell counts. Cells harvested from intrapulmonary/tracheobronchial (mediastinal) lymph nodes (LLN) (A) and auricular lymph nodes (ALN) (B) were counted using a Coulter counter. Data shown are $\pm$ SD ($n=6$). * $p<.05$ compared to non-sensitized (Non-sens.) mice challenged with saline (ANOVA). # $p<.05$ compared to non-sensitized mice challenged with 100 µg ATPC (ANOVA).

washing the plates, biotinylated rat anti-mouse IgE detection antibody (BD Pharmingen, San Diego, CA) was added to the wells and allowed to incubate at room temperature for 1 h. After washing to remove free detection antibody, 1.5 µg/mL streptavidin peroxidase (Zymed, Grand Island, NY) was added followed by incubation at 25 °C for 1 h. After washing, plates were developed by the addition of tetramethylbenzidine (TMB) substrate (Dako Corp., Glostrup, Denmark) and the reaction was terminated by the addition of H₂SO₄ (Fisher Scientific, Pittsburgh, PA). Optical density was determined at 450 nm using a SpectraMax 340 pc plate reader (Molecular Devices, Sunnyvale, CA). Mean IgE

concentrations were determined using Softmax Pro software (Molecular Devices, Sunnyvale, CA).

Total protein and LDH detection in BALF

LDH activity and total protein content of BALF were assessed using commercially available kits (Thermo Fisher Diagnostics, Waltham, MA) adapted for use with the Konelab 30 clinical chemistry analyzer (Thermo Clinical Lab Systems, Espoo, Finland) as described previously (Selgrade et al., 2006; Williams et al., 2015).

Statistical analysis

Statistical significance was defined as $p<.05$ as evaluated by one-way analysis of variance (ANOVA) and Bonferroni's multiple comparison test.

Results

Pulmonary responses to ATPC

Results from the murine local lymph node assay (Williams et al., 2014) and immediate type responses to platinum have previously been reported for mice dermally sensitized to AHCP (Williams et al., 2015). However, no other halogenated platinum compounds were tested using this mouse model. Consequently, it was not known if ATPC delivered to the respiratory tract would trigger respiratory responses in dermally sensitized mice. To address this uncertainty, BALB/c mice dermally sensitized to ATPC were challenged by IA instillation of ATPC (Figure 1). Consistent with previous findings (Basketter et al., 1999; Dearman et al., 1998, 2013; Williams et al., 2014), challenge with ATPC (100 µg) resulted in an increase in the number of lymph node cells present in the ALN (Figure 2(A); $p<.05$) and lung-associated lymph nodes (LLN) (Figure 2(B); $p<.05$) following an IA challenge on experimental day 24.

Immediately following IA instillation of ATPC or AHCP, respiratory responses were assessed by WBP. Average baseline Penh values were consistent across groups (at ~0.5 Penh; Figure 3(A)). An antigen-specific IR was evident in ATPC-sensitized mice following the IA challenge with 100 µg ATPC (Figure 3(A); $p<.05$) and in AHCP sensitized mice following 100 µg challenge with either AHCP or ATPC (Figure 3(C); $p<.05$), but there were no differences when comparing mice challenged with AHCP to mice challenged with ATPC.

Methacholine, a cholinergic agonist, is widely used to study nonspecific bronchial hyperreactivity. Two days after assessing the antigen-specific IR, respiratory responsiveness to increasing doses of Mch aerosol was evaluated. For ATPC sensitized mice challenged with 100 µg ATPC, a statistically significant increase in Penh was observed with 12.5 mg/mL inhaled Mch when compared to non-sensitized mice challenged with 100 µg ATPC (Figure 3(B); $p<.05$). Average Penh values were not significantly increased for non-sensitized mice challenged with saline or ATPC, or for

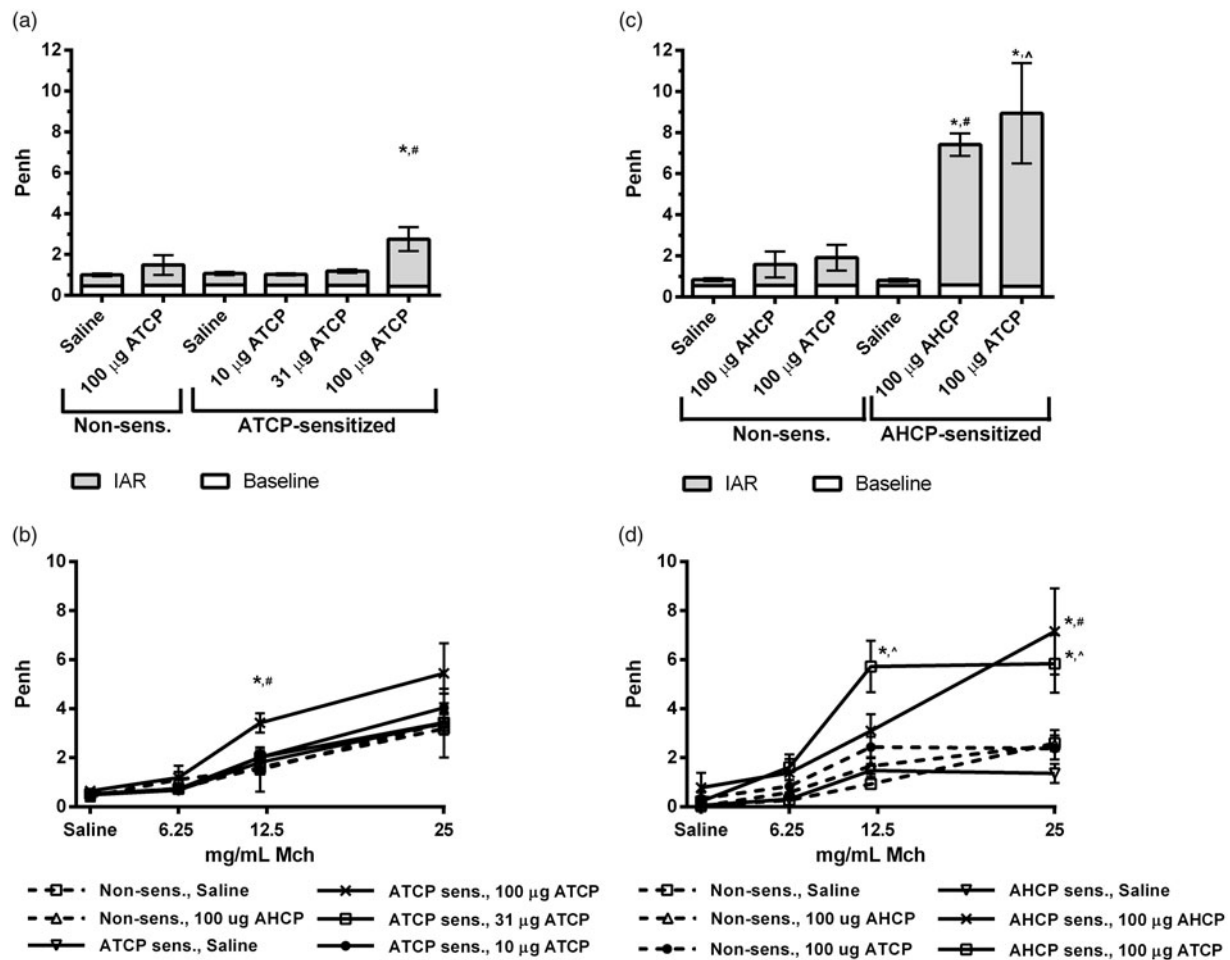


Figure 3. Pulmonary physiology. Pulmonary responses were measured using whole-body plethysmography. (A) ATCP-sensitized mice exhibit an immediate response (IR) following ATCP challenge on experimental day 24. (B) ATCP-sensitized mice challenged with ATCP exhibit a dose-dependent increase in methacholine (Mch) responsiveness on experimental day 26. (C) AHCP-sensitized mice challenged with ATCP exhibit an immediate response (IR) on experimental day 24. (D) AHCP-sensitized mice challenged with ATCP exhibit a dose-dependent increase in methacholine (Mch) responsiveness on experimental day 26. For all panels, data shown are $\pm$ SEM ($n = 6$). * $p < .05$ compared to non-sensitized mice challenged with saline (ANOVA). # $p < .05$ compared to non-sensitized mice challenged with 100 µg AHCP (ANOVA). ° $p < .05$ compared to non-sensitized mice challenged with 100 µg ATCP (ANOVA). Non-sens.: non-sensitized.

ATCP-sensitized mice challenged with saline (Figure 3(B)). For mice sensitized with AHCP and challenged with AHCP, a significant increase in Penh was observed with 25 mg/mL Mch, and, for those challenged with ATCP, a significant increase was observed at 12.5 mg/kg Mch ($p < .05$; Figure 3(D)).

Inflammatory cells infiltrate the airways of mice exposed to ATCP

BALF total cell counts in mice challenged with ATCP were significantly increased compared to mice challenged with saline (Figure 4(A,D); $p < .05$). The cellular composition of BALF collected from these mice was also affected by treatment. Most notably, the number of eosinophils, a marker of allergic inflammation (Akuthota et al., 2011), increased from 0.3% of the total BALF cells in non-sensitized mice challenged with 100 µg ATCP to $3.3 \pm 1\%$ in ATCP-sensitized mice challenged with 100 µg ATCP (Figure 4(B); $p < .05$). The proportion of neutrophils found in BALF was significantly elevated in both non-sensitized and ATCP-sensitized mice challenged with 100 µg ATCP (Figure 4(C); $p < .05$).

There were no statistically significant differences in the proportions of lymphocytes or macrophages in any experimental group (data not shown). Eosinophils and neutrophils were also elevated in mice sensitized with AHCP and challenged with either AHCP or ATCP ($p < .05$), but there were no statistical differences when comparing mice challenged with AHCP to mice challenged with ATCP (Figure 4(E,F)).

Biochemical markers of inflammation in response to ATCP

Non-specific lung inflammation was evaluated by measuring total protein and LDH and levels in cell-free supernatants of BALF. Irrespective of sensitization state, there were no statistically significant differences in total protein and LDH levels across groups of mice sensitized and challenged with ATCP (Figure 5(A,B)). Total protein levels were significantly increased in AHCP-sensitized mice challenged with AHCP ($p < .05$), but not when challenged with ATCP (Figure 5(C)). There were no statistically significant differences in LDH levels across groups of AHCP-sensitized mice challenged with either platinum compound (Figure 5(D)).

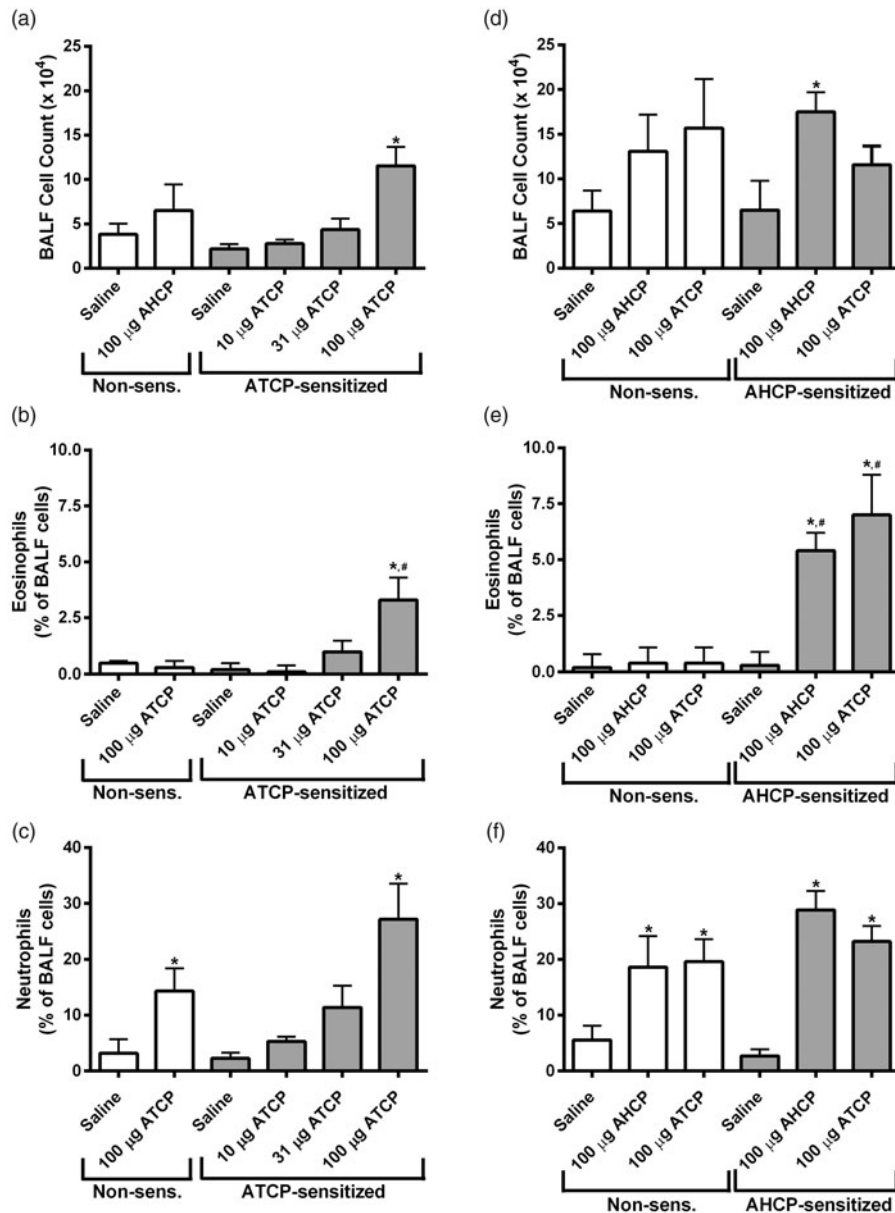


Figure 4. Lung instillation of AHCP results in cellular infiltration into BALF. (A) BALF was collected from non-sensitized and ATCP-sensitized mice on experimental day 26. Collected cells were enumerated using a Coulter counter. (B) Eosinophils selectively infiltrate the BALF of ATCP-sensitized mice following IA challenge. (C) Neutrophils infiltrated the BALF of non-sensitized and ATCP-sensitized mice challenged with AHCP. (D) BALF was collected from non-sensitized and AHCP-sensitized mice on experimental day 26. Collected cells were enumerated using a Coulter counter. (E) Neutrophils infiltrated the BALF of non-sensitized and AHCP-sensitized mice following IA challenge. (F) Eosinophils selectively infiltrate the BALF of AHCP-sensitized mice following IA challenge. For all panels, data shown are $\pm$ SEM ($n = 6$). * $p < .05$ compared to non-sensitized (Non-sens.) mice challenged with saline (ANOVA). # $p < .05$ compared to sensitized mice challenged with corresponding platinum compound (ANOVA). Non-sens.: non-sensitized.

Total serum IgE levels are elevated in ATCP-sensitized mice

Total serum IgE levels were dose-dependently increased in ATCP-sensitized mice compared to non-sensitized mice (Figure 6(A); $p < .05$). However, IgE levels were significantly lower in ATCP-sensitized mice challenged with saline (Figure 6(A); $p < .05$). Total serum IgE levels were also significantly elevated in AHCP-sensitized mice compared to non-sensitized mice. However, there were no dose-dependent differences between animals challenged with saline, AHCP or ATCP (Figure 6(B)).

Discussion

Recently, a mouse model of halogenated platinum salt sensitization was developed (Williams et al., 2015). Using this model, we showed that a single airway challenge with AHCP was capable of inducing changes in pulmonary function in dermally sensitized mice. In this study, we demonstrated that mice dermally sensitized with ATCP develop an immediate type hypersensitivity response following respiratory exposure to ATCP. The magnitude of the response was less than that for AHCP sensitized mice providing new evidence indicating that the degree of chlorination influences

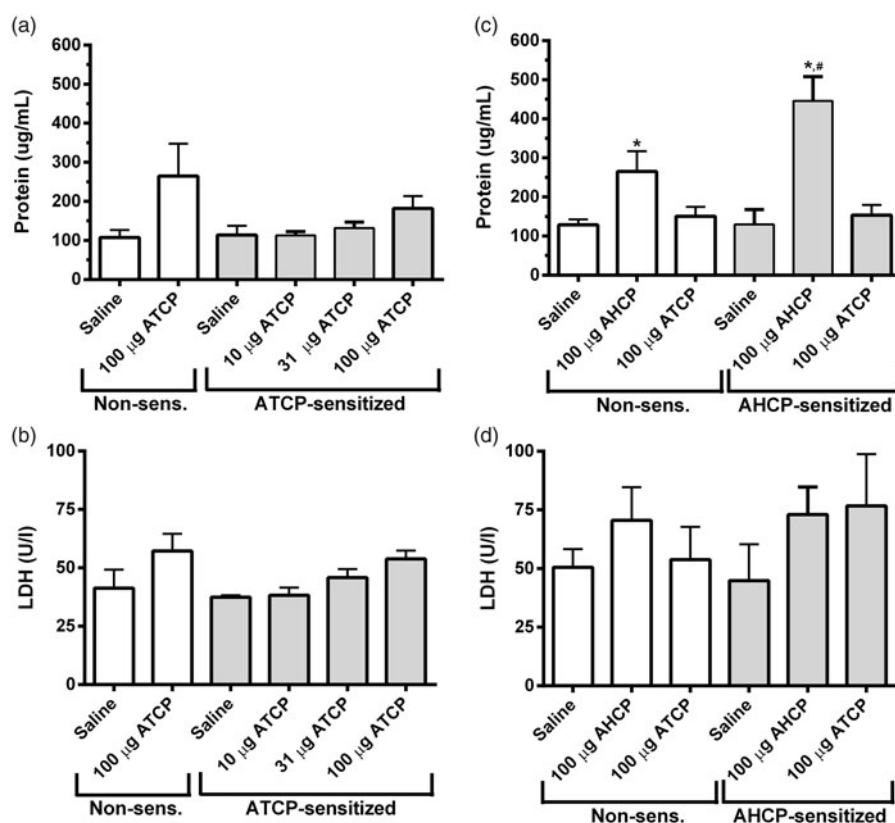


Figure 5. Protein levels are elevated in BALF collected from AHCP-challenged mice. Protein levels in BALF collected from ATCP-sensitized (A) and AHCP-sensitized (C) mice were determined by Coomassie blue. Lactate dehydrogenase levels in BALF collected from ATCP-sensitized (B) and AHCP-sensitized (D) mice were determined using Thermo LD-L Kit reagent and Thermo Trace Data-Trol controls. For all panels, data shown are $\pm$ SEM ($n = 6$). * $p < .05$ compared to non-sensitized (Non-sens.) challenged with saline (ANOVA). ** $p < .05$ compared to non-sensitized mice challenged with 100 μ g AHCP (ANOVA). Non-sens.: non-sensitized.

sensitization potency. We also show for the first time that mice dermally sensitized to AHCP exhibit allergic cross-reactivity responses following airway challenge with a second platinum compound, ATCP.

Allergic sensitization to ATCP has been demonstrated by positive skin prick tests in platinum refinery workers (Pepys et al., 1979). However, relatively few laboratory-based studies have been performed on this platinum compound (Basketter et al., 1999; Cleare et al., 1976; Dearman et al., 1998, 2013; Murdoch & Pepys, 1984b; Williams et al., 2014) and none of those reports assessed changes in lung function following targeted delivery of platinum to the airways. Consequently, very little is known about the allergic potential and respiratory effects of this halogenated platinum salt.

In this study, mice topically sensitized to ATCP exhibited responses characteristic of the immediate type hypersensitivity including: (1) detection of an IR and eosinophilic infiltration in the lungs of sensitized mice following respiratory tract challenge with ATCP, (2) increased responsiveness to nonspecific stimuli (i.e. Mch) and (3) significantly elevated levels of total serum IgE. For most parameters measured (i.e. protein and LDH levels in BALF, neutrophil and eosinophil lung infiltration and total serum IgE levels), topical sensitization with ATCP produced similar responses to those previously reported in AHCP sensitized mice (Williams et al., 2015). These data indicate that dermal exposure to ATCP induces immediate type hypersensitivity and that pulmonary responses can be triggered by

respiratory challenge, but questions remain regarding the allergic potency of ATCP relative to AHCP.

The sensitization potential of halogenated platinum salts has been attributed to the number of chlorines coordinated with platinum (Cleare et al., 1976; Linnett & Hughes, 1999; Murdoch & Pepys, 1984a,b, 1985, 1986; Schuppe et al., 1992, 1997). However, data from the LLNA suggest that AHCP, ATCP and cisplatin, each with a different number of chlorines coordinated with platinum, are similar in their ability to sensitize mice via the dermal route (Williams et al., 2015). However, none of the aforementioned studies investigated the physiological consequences of platinum sensitization. Taking advantage of our mouse model, we are the first to assess functional endpoints observed in mice sensitized to different platinum compounds. Compared to the effect observed with AHCP (Table 1), the IR precipitated by challenge with 100 μ g ATCP was notably less dramatic than the effect observed with 100 μ g AHCP. Not only was the magnitude of the IR less in ATCP-sensitized/challenged mice, but an IR was evident in AHCP sensitized mice challenged a dose nearly three times lower than that required to illicit an IR in ATCP-sensitized mice. Additionally, based on data for sensitized mice challenged with saline, our data suggest that the circulating pool of IgE is more stable following sensitization with AHCP than ATCP, which may contribute to differences in the strength of the allergic response. Together, these data suggest that AHCP is a more potent inducer of pulmonary responses than ATCP, an

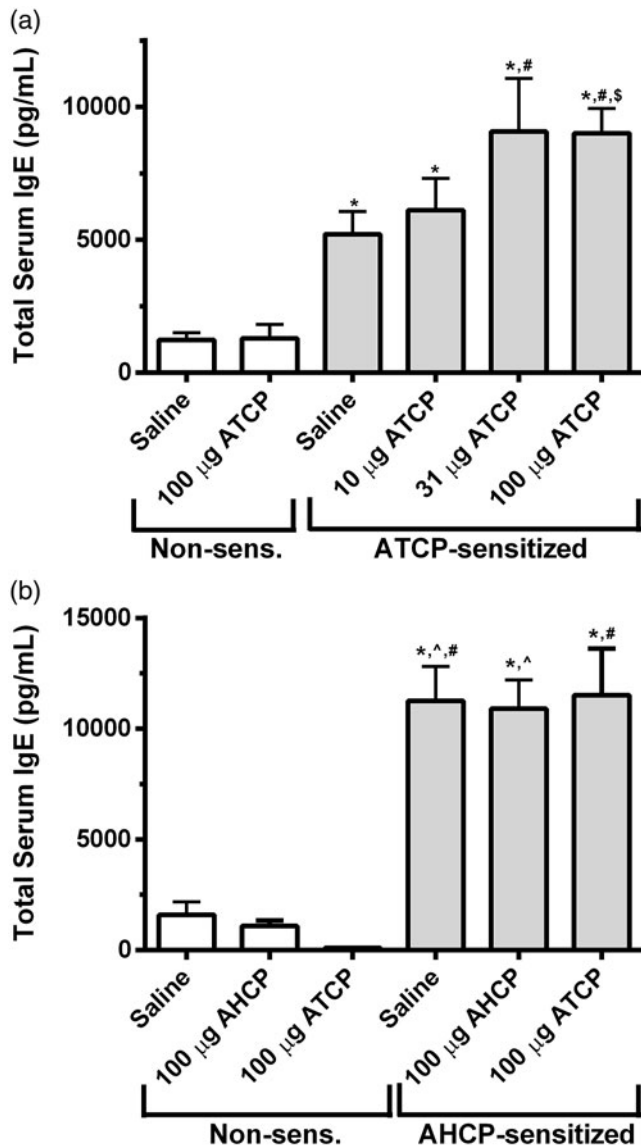


Figure 6. Total serum IgE levels were significantly elevated in mice sensitized to AHCP. Total serum IgE levels from ATCP-sensitized (A) and AHCP-sensitized (B) mice were determined by ELISA on experimental day 26. For all panels, data shown are $\pm$ SEM ($n = 6$). * $p < .05$ compared to non-sensitized (Non-sens.) mice challenged with saline (ANOVA). # $p < .05$ compared to non-sensitized mice challenged with 100 µg ATCP (ANOVA). ^ $p < .05$ compared to non-sensitized mice challenged with 100 µg AHCP (ANOVA). \$ $p < .05$ compared to non-sensitized mice challenged with 100 µg ATCP (ANOVA). Non-sens.: non-sensitized.

Table 1. Summary data and comparisons to previously published study with AHCP.

	From Williams et al. (2015)		Current study	
			AHCP sensitized	
	AHCP sensitized Challenged with 100 µg AHCP	ATCP sensitized Challenged with 100 µg ATCP	Challenged with 100 µg AHCP	Challenged with 100 µg ATCP
IR (Penh; $\pm$ SEM)	9.2 (0.6)	2.3 (0.6)	6.8 (0.6)	8.4 (2.4)
Response to 25 mg/mL Mch (Penh; $\pm$ SEM)	5.3 (0.6)	5.5 (1.2)	7.2 (1.8)	5.8 (1.2)
Total BALF cell count ($\times 10^4$, $\pm$ SEM)	16.8 (2.2)	11.6 (2.1)	17.5 (2.2)	11.6 (2.1)
% Eosinophils ($\pm$ SEM)	5.1% (1.4)	3.3 (1.0)	5.4 (0.8)	7.0 (0.8)
% Neutrophils ($\pm$ SEM)		27.2 (6.4)	28.9 (3.4)	23.2 (2.8)
BAL protein ($\pm$ SEM)	474.2 (87.0)	181.6 (31.9)	445.6 (62.0)	153.8 (26.0)
BAL LDH ($\pm$ SEM)	75.8 (9.9)	53.8 (3.6)	70.6 (14.2)	76.7 (22.1)
Total serum IgE ($\pm$ SEM)	19,204.5 (2376.8)	9018.7 (927.0)	10,909.9 (1296.2)	11,519.9 (2100.6)
LLN counts ($\pm$ SD)	1.9 (0.2)	2.3 (0.2)	ND	ND
ALN counts ($\pm$ SD)	4.1 (0.1)	5.5 (0.5)	ND	ND

AHCP: ammonium hexachloroplatinate; ATCP: ammonium tetrachloroplatinate; IR: antigen-specific immediate response; SEM: standard error of the mean; Mch: methacholine; BAL: bronchoalveolar lavage; LDH: lactate dehydrogenase; IgE: immunoglobulin E; LLN: lung-associated lymph nodes; ALN: auricular lymph nodes; ND: not determined.

outcome that is consistent with the suggestion that the number of chlorines coordinated with platinum drives sensitization potential. Furthermore, these results suggest that LLNA potency values may not be predictive of functional endpoints when dermally sensitized mice are challenged by targeted delivery to the airways. Additional work is necessary to more conclusively determine whether, or not the degree of chlorination influences manifestation of allergic effects in topically exposed mice.

Cross-reactivity between halogenated platinum salts has been reported (Murdoch & Pepys, 1985), but an animal model was not previously available to assess changes in lung function (Williams et al., 2015). Consequently, it was not known if cross-reactivity would result in functional consequences. For the first time, we demonstrate that mice topically sensitized to AHCP cross-react after a single airway challenge with ATCP. This conclusion is supported by several lines of evidence including (1) utilization of high-purity platinum salts (i.e. $\geq 99\%$), virtually eliminating trace metals contaminants, for these experiments, (2) detection of an IR and eosinophilic infiltration in the lungs of AHCP-sensitized mice following a single intra-airway challenge with ATCP, (3) the airways of AHCP-sensitized mice challenged with ATCP are responsive to nonspecific stimuli (i.e. Mch) and (4) positive control data from parallel treatment groups demonstrating that the mice were in fact sensitized to AHCP. Not only did we detect strong evidence of cross-reactivity, but our data also show significant magnification of the allergic response. Compared to ATCP-sensitized and challenged mice, intra-airway challenge of AHCP-sensitized mice with ATCP resulted in a dramatic increase in average Penh from 2.31 ± 0.59 to 8.41 ± 2.4 . In addition, infiltration of the lungs by eosinophils was also greater in AHCP-sensitized mice challenged with ATCP ($7.0 \pm 1.8\%$) than in ATCP-sensitized/challenged mice ($3.3 \pm 1\%$). We speculate that exacerbation of ATCP-induced effects may be related to the larger pool of serum IgE in AHCP-sensitized mice on day 24. Taken together, these data indicate that dermal exposure to AHCP induces the development of immediate type hypersensitivity and that respiratory challenge with a second, different platinum compound is sufficient to trigger pulmonary responses.

Conclusions

Using a mouse model of platinum salt sensitization, we confirmed the functional consequences of dermal sensitization with AHCP and, for the first time, demonstrated that dermal exposure to ATPC can lead to changes in pulmonary function following airway challenge. In addition, we provide evidence for cross-reactivity among halogenated platinum salts including the only published demonstration that cross-reactivity among platinum salts alters pulmonary function. Continued use of this model will provide insights into the mechanism and consequences of platinum hypersensitivity necessary for informing human health risk assessment.

Acknowledgements

The authors thank C. Copeland, L. Copeland, D. Andrews and J. Richards for their expert technical assistance. We also thank Drs. MJ. Selgrade, A. Farraj and I. Gilmour for their thoughtful and critical review of this work.

Disclosure statement

This article has been reviewed by the U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency or of the US Federal Government, nor does the mention of trade names or commercial products constitute endorsement or recommendations for use of those products. The authors report no financial or other conflicts of interest. The authors alone are responsible for the content and writing of this article.

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