

Acrolein Induction of Oxidative Stress and Degranulation in Mast Cells

Daniel J. Hochman, Christopher R. Collaco, Edward G. Brooks

Department of Pediatrics, University of Texas Medical Branch, 301 University Blvd.,
Galveston, Texas 77555-0369

Received 26 July 2012; revised 1 September 2012; accepted 10 September 2012

ABSTRACT: Increases in asthma worldwide have been associated epidemiologically with expanding urban air pollution. The mechanistic relationship between airway hyper-responsiveness, inflammation, and ambient airborne triggers remains ambiguous. Acrolein, a ubiquitous aldehyde pollutant, is a product of incomplete combustion reactions. Acrolein is abundant in cigarette smoke, effluent from industrial smokestacks, diesel exhaust, and even hot oil cooking vapors. Acrolein is a potent airway irritant and can induce airway hyper-responsiveness and inflammation in the lungs of animal models. In the present study, we utilized the mast cell analog, RBL-2H3, to interrogate the responses of cells relevant to airway inflammation and allergic responses as a model for the induction of asthma-like conditions upon exposure to acrolein. We hypothesized that acrolein would induce oxidative stress and degranulation in airway mast cells. Our results indicate that acrolein at 1 ppm initiated degranulation and promoted the generation of reactive oxygen species (ROS). Introduction of antioxidants to the system significantly reduced both ROS generation and degranulation. At higher levels of exposure (above 100 ppm), RBL-2H3 cells displayed signs of severe toxicity. This experimental data indicates acrolein can induce an allergic inflammation in mast cell lines, and the initiation of degranulation was moderated by the application of antioxidants.

© 2012 Wiley Periodicals, Inc. *Environ Toxicol* 00: 000–000, 2012.

Keywords: acrolein; oxidative stress; mast cell; degranulation; cellular toxicity; air pollution; cytokines; allergic inflammation

INTRODUCTION

Acrolein, a major constituent of combustion products and tobacco smoke, is best known as a carcinogen and produces protein and DNA adducts in high concentration exposures (Liu et al., 2010; Zhang et al., 2011). For example, acrolein induces G to T and G to A mutations forming mutagenic

α - and γ -hydroxy-1, *N*(2)-cyclic propano-2'-deoxyguanosine adducts. These adducts enhance oxidative DNA damage-induced mutagenesis, inhibit DNA repair proteins (Wang et al., 2012), and may contribute to p53 mutations in lung cancer (Feng et al., 2006).

Asthma is a disease on the rise around the world. Exacerbations of asthmatic episodes are associated with increases in air pollution through an abundance of epidemiological data (Samoli et al., 2011). Asthma is typically characterized by a chronic state of inflammation combined with hyper-responsive airway constriction (Hargreave and Nair, 2009). However, the physiological relationship between asthma and air pollution remains elusive.

Acrolein is also a known respiratory irritant (Ghilarducci and Tjeerdema, 1995). Airborne aldehydes have been implicated as contributing factors to the increase in asthma and allergic disease in world urban populations (Leikauf,

Correspondence to: E. G. Brooks; e-mail: brookse@uthscsa.edu

Contract grant sponsor: the Sealy Center for Environmental Health and Medicine and by the National Institutes of Environmental Health Sciences Center at the University of Texas Medical Branch, Galveston, Texas

Contract grant number: P01-ES06676

Contract grant sponsor: National Institute of Environmental Health Sciences

Contract grant number: T32-07524

Published online in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/tox.21818

© 2012 Wiley Periodicals, Inc.

2002; McGwin et al., 2010; Arif and Delclos, 2012). In animal models, acrolein induces airway hyper-responsiveness (Myou et al., 1993), production of leukotrienes (Leikauf et al., 1989), increased mucous production (Borchers et al., 1998), and airway hypersensitivity (Roux et al., 1999), all hallmarks of asthma. Thus, as one of the more common components of anthropogenic pollution, acrolein poses a potential health risk for the development of asthma in exposed populations.

Airway mast cells release inflammatory mediators, such as histamine, cytokines, and leukotrienes, responsible, in part, for the initiation of allergic inflammation (Fireman, 2003). It is thought that oxidative stress may contribute to the release of these mediators (Li et al., 2003). Previously, we have shown that sodium sulfite, the soluble form of sulfur dioxide, induces intracellular oxidant stress and degranulation in mast cells (Collaco et al., 2006).

In this study, we sought to investigate the effects of acrolein on mast cell function. We hypothesized that acrolein acts directly on mast cells to induce the release of inflammatory mediators and production of intracellular reactive oxygen species (ROS).

METHODS

Reagents

Bovine serum albumin (BSA), *N*-acetyl-L-cysteine (NAC), tetramethylthiourea (TMTU), diphenyliodonium (DPI), 2',7'-dichlorofluorescein diacetate (DCFH-DA), Acrolein (C_3H_4O), and mouse IgE anti-dinitrophenyl (DNP-IgE) antibodies were purchased from Sigma-Aldrich (St Louis, MO). Dinitrophenyl coupled to BSA (DNP) was purchased from Biosearch Technologies (Novato, CA). Dulbecco modified eagle medium (DMEM), fetal calf serum (FCS), streptomycin-penicillin, and Hanks balanced salt solution (HBSS) were purchased from Gibco BRL (Grand Island, NY).

Cell Culture

RBL-2H3 cells were obtained from American Type Cell Collection (ATCC) and cultured in DMEM supplemented with 10% FCS, 100 IU/mL penicillin, and 100 μ g/mL streptomycin (Gibco BRL, Grand Island, NY) at 37°C in a humidified atmosphere with 5% CO_2 .

Mast Cell Degranulation

As described in a recent review (Rashid et al., 2012), RBL-2H3 cells were established as an efficient and reliable alternative to the wild-type mast cell in inflammation research. These experiments were performed as described previously (Collaco et al., 2006). Briefly, RBL-2H3 cells were plated

at 1×10^4 per well in a 96-well plate in DMEM supplemented with 5% FCS and 1 μ Ci/mL of [H^3]serotonin overnight. Cells were then washed with HBSS, followed by application of experimental conditions. After incubation, supernatants were harvested, and any cells present were removed by centrifugation. Total cellular content of serotonin was quantified in the supernatant of cells lysed with Triton X100. Radioactivity was measured by scintillation spectroscopy (1450 MicroBeta; PerkinElmer, Fremont, CA) and the percentage release calculated: (experimental-spontaneous)/(total-spontaneous). For calcium depletion experiments, cells were labeled with [H^3]serotonin and then washed and assayed with calcium free HBSS plus 1 mM EDTA.

Determination of Intracellular ROS Generation

RBL-2H3 cells were washed with phosphate-buffered saline (PBS) before loading with 50 μ M DCFH-DA in PBS for 20 min at 37°C. The cells were then washed with PBS, incubated in experimental conditions and immediately placed into an automated fluorometer (FLUOstar Optima, BMG Labtech, Durham, NC), with an excitation wavelength of 480 nm and emission of 530 nm. The antioxidants, TMTU (50 μ M) and NAC (10 mM) were prepared in PBS and mixed with and without acrolein before adding to wells. In experiments using DPI, cells were incubated with 100 μ M DPI for 30 min at 37°C, followed by two washes with PBS before DCFH-DA loading. The inhibitors themselves did not induce fluorescence. Results were calculated by subtracting spontaneous fluorescence in wells containing PBS alone and are expressed in relative fluorescence units.

Cellular Viability Measurements

Cell viability was evaluated using several methods. Propidium iodide (PI) (Calbiochem, San Diego, CA) staining was used to assess cellular membrane integrity. Cells were incubated for 30 min with acrolein. The cells were then harvested with trypsin plus 0.1% EDTA, washed twice with PBS with 0.5% BSA, and stained with PI (1 μ g/mL) in PBS with 0.5% BSA. Fluorescence was evaluated by flow cytometry (FACScan; Becton Dickinson, San Jose, CA).

A second method of analyzing cell viability used the reduction of tetrazolium dye to assess mitochondrial activity as an indication of cell health. The MTT assay was performed according to manufacturer's protocols (Cayman, Ann Arbor, MI). Briefly, cells were incubated with acrolein for 30 min. Cells were washed with PBS/10% FCS. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added to the cells for 4 h at 37°C. The supernatant was removed and replaced with isopropanol and the optical density (540 nm) read in an automated plate reader (FLUOstar Optima).

A third method for assessing cellular viability is known as the JAM ("just another method") assay (Matzinger, 1991) and assesses the amount of intact nonfragmented DNA within cells. RBL-2H3 cells were incubated with 1 $\mu\text{Ci/mL}$ ^3H -thymidine overnight. Cells were washed and incubated with acrolein. After incubation, cells were harvested on a semiautomated harvester and DNA deposited onto glass fiber filters, which were dried and placed into scintillation fluid and counts enumerated by scintillation spectroscopy. Total raw counts were compared between experimental conditions.

Intracellular Calcium Measurement

Intracellular calcium was determined using the FLIPR Calcium 3 Assay Kit (Molecular Devices, Sunnyvale, CA), which utilizes a calcium sensitive fluorescent dye, according to manufacturer's instructions. Briefly, RBL-2H3 cells were grown to confluency on 96-well plates. Medium was removed and 100 μL of the FLIPR Calcium 3 assay reagent in HBSS +20 mM Hepes was placed into wells and the plate incubated at 37°C for 1 h. After placing varying concentrations of acrolein, the plate was immediately placed into an automated microplate reader (FluoStar Optima) and fluorescence determined (excitation 480 nm, emission 530 nm). Results were calculated as a ratio with spontaneous fluorescence in wells containing PBS alone.

Cytokine, Chemokine, and Leukotriene Assays

RBL-2H3 cells were sensitized with DNP-IgE (200 ng/mL) for 1 h, washed, and then incubated with/without DNP and with/without acrolein and incubated for 30 min at 37°C . Cells were then washed and allowed to incubate another 24 h at 37°C . Culture supernatants were harvested and immediately frozen at -20°C until analysis. Cytokines were measured using a rat 9-plex: IL-1 α , IL-1 β , IL-2, IL-4, IL-6, IL-10, GM-CSF, IFN γ and TNF α (Bio-Rad, Hercules, CA) and read on a Bio-Plex system (Bio-Rad) according to the manufacturer's instructions. The assay performance reports a detection limit of <10 pg/mL with a dynamic range of 0.2–32,000 pg/mL. 14,15-leukotriene C₄ (LTC₄) levels were assessed using an EIA according to manufacturer's instructions (Cayman) and optical density read (520 nm) on an automated plate reader (FLUOstar Optima).

Statistical Analyses

All analyses were performed on the means of three individual experiments, and experimental samples were performed in duplicate. Degranulation was analyzed using the Spearman correlation method and individual comparisons were

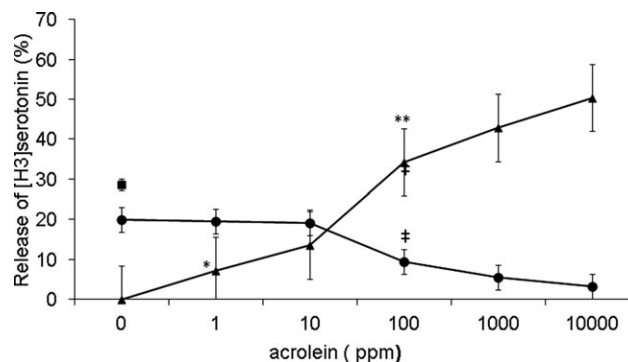


Fig. 1. Degranulation with and without IgE crosslinking. Release of [^3H] serotonin in RBL-2H3 cells increases with increasing concentrations of acrolein ($\blacktriangle$). Cells were also sensitized to DNP-IgE and serotonin release stimulated with either an optimal (50 ng/mL; $\blacksquare$) or suboptimal (1 ng/mL, $\circ$) concentration of DNP. Addition of increasing concentrations of acrolein inhibits release by suboptimal DNP. Values represent means ($\pm$ SEM) of a minimum of three independent experiments. *Denotes statistical significance between negative control and 1 ppm acrolein ($p < 0.05$). **Denotes statistical significance between 1 and 100 ppm acrolein ($p < 0.05$). ‡Denotes statistical significance between 1 ng/mL DNP (no acrolein) and 1 ng/mL DNP + 100 ppm acrolein ($p < 0.05$).

made using a paired 2-sample for means t test was used, with a 95% confidence interval ($p < 0.05$).

RESULTS

Acrolein Induces Mast Cell Degranulation

In experiments to detect the direct effect of acrolein on mast cell degranulation, RBL-2H3 cells exposed to acrolein induced a progressive increase in the release of [^3H]serotonin from 1–10,000 ppm (Fig. 1). Subsequently, we investigated the potential ability of acrolein to synergize with IgE crosslinking. To accomplish this, RBL-2H3 cells were sensitized with DNP-IgE (200 ng/mL) for 1 h, washed and then exposed to DNP with or without acrolein. Using concentrations of DNP (1 ng/mL), that induced suboptimal degranulation, there was suppression of serotonin release at acrolein concentrations of 100–10,000 ppm (Fig. 1). This suggested alternative mechanisms existed for acrolein-induced degranulation versus IgE-crosslinking.

To further explore acrolein-induced degranulation mechanisms, it has been shown previously that IgE-dependent mast cell degranulation is dependent on the influx of extracellular calcium (Lee and Oliver, 1995; Oka et al., 2002). However, we have shown previously that degranulation by a strong oxidant stimulus occurred in the absence of extracellular calcium (Collaco et al., 2006). Thus, intracellular calcium release in RBL-2H3 cells exposed to acrolein was

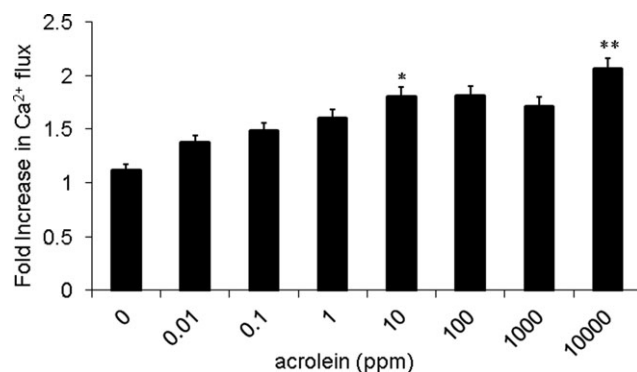


Fig. 2. Intracellular Ca^{2+} flux. RBL-2H3 cells were treated with a calcium sensitive dye (FLPR system) and immediately read after placing concentrations of acrolein onto the wells. Values represent mean fold increases ($\pm$ SEM) over cell-free wells ($n = 3$ experiments). Results show a steady rise in Ca^{2+} release with increasing concentrations of acrolein. *Indicates statistically significant increase from wells with no acrolein ($p < 0.05$, $n = 3$). **Indicates statistical significance between 10 and 10,000 ppm acrolein ($p < 0.05$, $n = 3$).

determined. Calcium levels increased at 0.01 ppm acrolein, and demonstrated a statistically significant increase by 10 ppm (Fig. 2). To determine if degranulation was dependent upon the influx of extracellular calcium, serotonin release was determined after extracellular calcium depletion (Fig. 3), which demonstrated no effect. Thus, we concluded that calcium stores from intracellular sources may be sufficient to induce degranulation in response to acrolein, a distinct difference from the mechanism reported previously for IgE-mediated degranulation (Lee and Oliver, 1995).

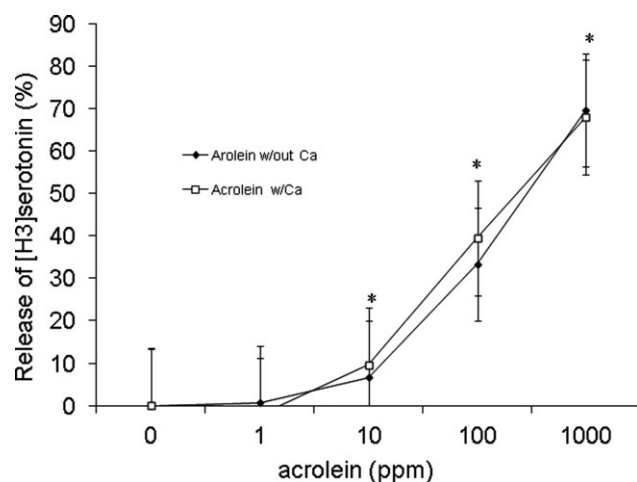


Fig. 3. Degranulation after extracellular calcium depletion. RBL-2H3 cells were incubated with [H^3]serotonin and then placed into calcium-depleted HBSS containing 1 mM EDTA, prior to exposure to varying concentrations of acrolein. The results demonstrate no significant differences between calcium depleted and nondepleted cells.

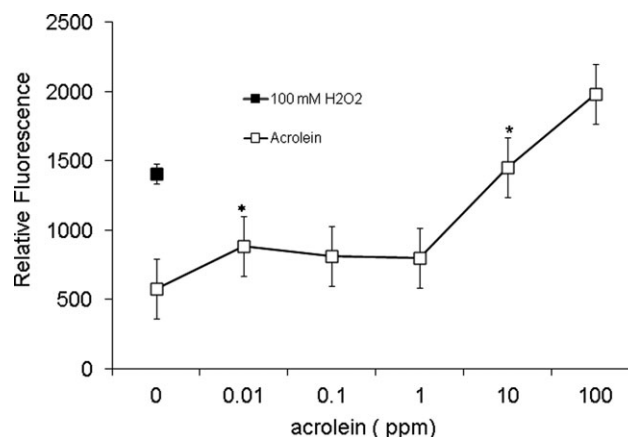


Fig. 4. Intracellular ROS generation. RBL-2H3 cells were labeled with DCFH-DA and treated with either PBS or acrolein. ROS increases initially at 0.01 ppm and to an equivalent level as 100 mM H_2O_2 at 10 ppm. Data are mean levels ($\pm$ SEM) from 3 independent experiments. * Indicates statistical significance between the indicated concentration and the previous concentration ($p < 0.05$).

Acrolein Induces Generation of Reactive Oxygen Species

In previous studies, the generation of intracellular ROS was associated with mast cell activation and degranulation

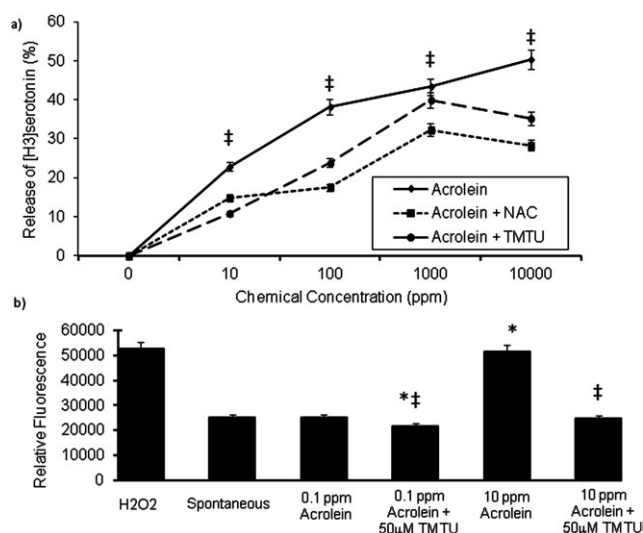


Fig. 5. Antioxidant treatment effects on degranulation and ROS levels. (a) RBL-2H3 cells treated with TMTU (50 μM) and NAC (10 mM) had reduced levels of [H^3] serotonin release induced by acrolein. (b) RBL-2H3 cells treated with TMTU (50 μM) had reduced levels of DCF fluorescence induced by acrolein. * indicates statistical differences from spontaneous fluorescence ($p < 0.05$, $n = 3$). ‡ indicates statistical difference from cells without antioxidant ($p < 0.05$, $n = 3$).

TABLE I. Cytokine production in RBL-2H3 cells exposed to acrolein

	IL-1 α	IL-1 β	IL-2	IL-4	IL-6	IL-10	IFN γ	TNF α	GM-CSF
Acrolein 10,000 ppm	61 ^a	160	49	10	903	460	128	241	41
+ DNP 0.1	98	366	118	18	1594	853	231	415	85
+ DNP 50	136	509	170	22	2167	1184	292	558	107

^a Results are expressed as pg/mL culture supernatant.

(Collaco et al., 2006). Thus, we sought to investigate the mechanism of degranulation by determining the generation of intracellular ROS after acrolein exposure. Using an ROS sensitive fluorescent dye, DCFH-DA, acrolein exposed RBL-2H3 cells showed significant increases in intracellular ROS with increasing acrolein concentrations. Intracellular ROS levels increased modestly but significantly with 0.01 ppm and more substantially at 10 ppm, which was equivalent to ROS levels after exposure to 100 mM H₂O₂ (Fig. 4). To investigate further, we treated cells with ROS scavengers, TMTU or NAC, which significantly reduced both ROS production and serotonin release (Fig. 5). To investigate if flavoenzymes, such as those located within NADPH oxidase or mitochondrial complex II were involved in ROS generation by acrolein, cells were treated with 100 μ M DPI prior to exposure to acrolein. No change in serotonin release was observed (data not shown).

Acrolein Induces Expression of Inflammatory Cytokines

An additional indicator of mast cell activation is the secretion of inflammatory mediators, which may occur with or without degranulation (Theoharides et al., 2007). We measured the presence of cytokines in culture supernatants of RBL-2H3 cells activated with increasing concentrations of acrolein and with or without DNP-IgE/DNP. Elevated levels of cytokines (Table I) were detected only after exposure of RBL-2H3 cells to higher concentrations (10,000 ppm) of acrolein (Table I). All other concentrations of acrolein yielded undetectable or near undetectable levels. However, in contrast to degranulation experiments, there was an enhancement of cytokine release in combination with IgE crosslinking to low (1 ng/mL) and high (50 ng/mL) concentrations of DNP. Levels of LTC₄ were only increased with acrolein at 1000 ppm (520 μ g/mL) and 10,000 ppm (1108 μ g/mL) as compared to 2380 μ g/mL with DNP-IgE/DNP (50 ng/mL) alone.

Acrolein Induces Cellular Toxicity in Mast Cells

Acrolein is known to have significant cellular toxicities. To investigate the level of toxicity in our system, several methods were utilized. The MTT assay (Fig. 6) assesses the ability of cells with functional mitochondria to reduce a tetra-

zolium dye and is routinely used as an assessment of viability and/or cell growth. Increasing doses of acrolein incubated with RBL-2H3 cells for 30 min caused a dose-dependent loss in the ability of the cells to reduce the dye, beginning at 10 ppm and significantly at 100 ppm. This can be interpreted as loss of cellular viability and/or a loss in mitochondrial function.

PI stains the DNA of cells whose membrane integrity is compromised. To assess membrane integrity, RBL-2H3 cells were treated with acrolein for 30 min, stained with PI and analyzed by flow cytometry (Fig. 7). At 10 ppm acrolein, few cells stained with PI, but at doses of 100 ppm and greater, increasing percentages of cells demonstrated positive staining. These data imply a significant loss of membrane integrity at 100 ppm acrolein.

The JAM assay measures the loss of prelabeled DNA from cells due to the fragmentation of DNA from apoptotic cells and is considered a sensitive measure of apoptotic cell death (Matzinger, 1991). RBL-2H3 cells treated with acrolein for 30 min demonstrated a modest loss (20%) of DNA at acrolein concentrations of 1–100 ppm, while higher doses showed a progressive increase in loss up to 80% at 10,000 ppm (Fig. 8). These experiments suggested that DNA fragmentation may occur at low levels (1 ppm) but

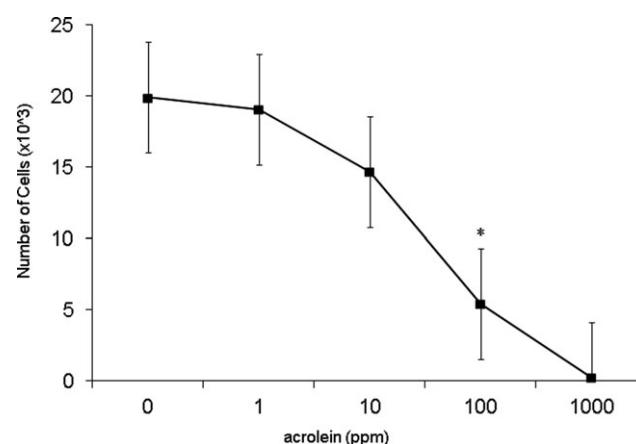


Fig. 6. Assessment of cellular viability-tetrazolium dye reduction. MTT assay assessing cellular viability in RBL-2H3 cells treated with increasing concentrations of acrolein for 30 min. Mitochondrial viability decreases substantially at 100 ppm. Values represent means ($\pm$ SEM). *Indicates statistical difference from previous lower concentration ($p < 0.05$, $n = 3$).

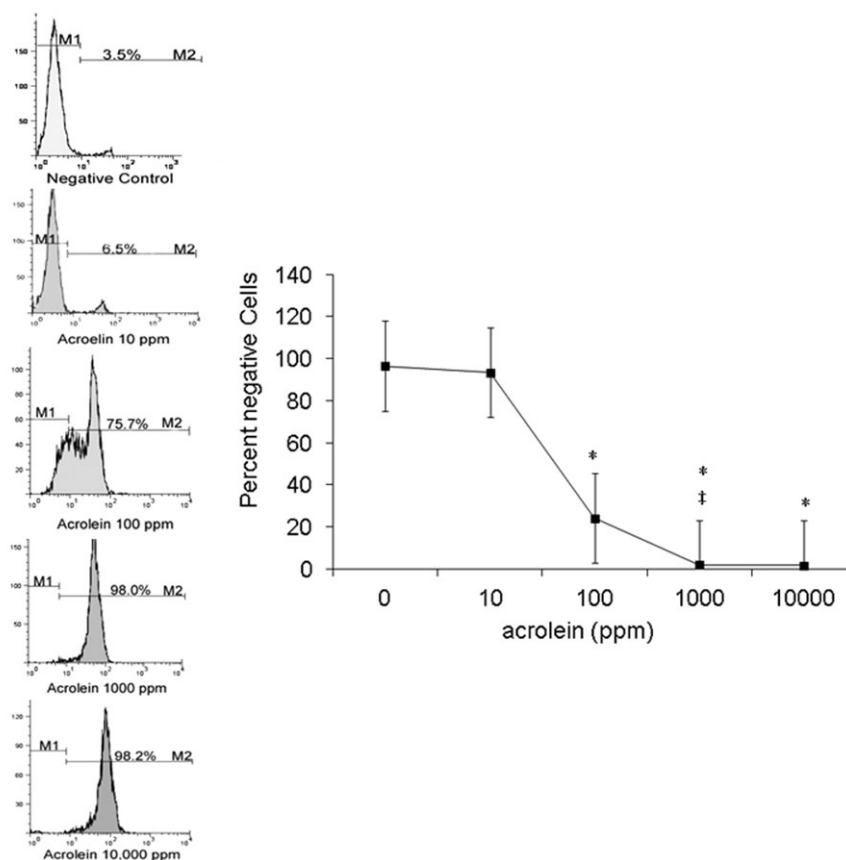


Fig. 7. Assessment of membrane integrity-propidium iodide staining. Flow cytometry of RBL-2H3 cells exposed to increasing doses of acrolein for 30 min and stained with propidium iodide by flow cytometry. A dramatic drop in cell viability occurs at 100 ppm acrolein exposure. Values represent means ($\pm$ SEM). “*” indicates statistical difference from negative control ($p < 0.05$, $n = 3$). † indicates statistical difference from previous concentration ($p < 0.05$, $n = 3$).

that substantial cell death and DNA fragmentation does not occur until much higher levels (>100 ppm). Together these data indicate that acrolein is toxic to mast cells beginning at concentrations as low as 1 ppm, but that significant cell death/mitochondrial dysfunction, increased membrane permeability and loss of DNA occurs at concentrations of 100–1000 ppm.

DISCUSSION

Acrolein is a major component of urban air pollution. It is released in emissions and effluents from manufacturing, combustion processes (including tobacco smoking and combustion of petrochemical fuels), from direct application to water and waste water as a slimicide and aquatic herbicide, as a photo-oxidation product of various hydrocarbon pollutants found in air (including propylene and 1,3-butadiene), and from land disposal of organic waste materials (Howard, 89 A.D.; Ghilarducci and Tjeerdema, 1995; Shaughnessy et al., 2001).

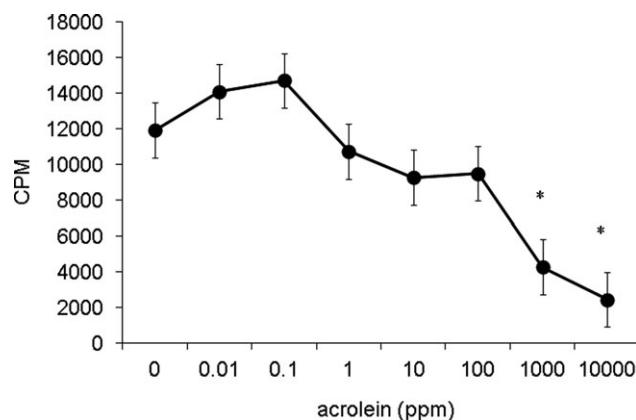


Fig. 8. Loss of cellular DNA-JAM assay. RBL-2H3 cells were pulsed with [H^3] thymidine and treated with increasing doses of acrolein for 30 min. Cell growth rates were measured via retention of incorporated [H^3] thymidine. A statistically significant drop in DNA fragmentation is shown at 1000 ppm acrolein exposure. Values represent means ($\pm$ SEM). “*” Indicates statistical difference between indicated concentration and negative control ($p < 0.05$, $n = 3$).

Epidemiologic studies have shown the physiologically relevant range of acrolein exposure is between 0.5 and 50 ppm. The average daily exposure is estimated in the range of 0.5–1.5 ppm (Caldwell et al., 1998; Woodruff et al., 1998; Tam and Neumann, 2004). The results of our experiments show a bimodal response of mast cells to acrolein exposure. High levels of exposure (>100 ppm) lead to cellular toxicity, high levels of ROS and release of mast cell inflammatory mediators, while lower levels (1–10 ppm), resulted in no toxicity nor leukotriene or cytokine release, but substantial ROS generation and degranulation. While the lowest concentrations of acrolein (<0.5 ppm) had little effect on mast cell degranulation, there was evidence for early expression of ROS and calcium release beginning at 0.01 ppm, thus, heralding the initial activation of mast cell proinflammatory pathways.

Our data demonstrating cytokine and leukotriene release at the highest levels of acrolein exposure, may be relevant in very high exposures such as might occur from direct exposure to tobacco smoke, wood fires, or acrolein processing (Ghilarducci and Tjeerdema, 1995; Shaughnessy et al., 2001). In this situation, it is likely that release of preformed cytokines resulted from the loss of cellular membrane integrity rather than active secretory processes. The toxicity we observed may be similar to that seen in previous studies (Rahman and MacNee, 2000; Ranganna et al., 2002), where acrolein was shown to induce toxicity through the depletion of GSH and activation of MAP kinases leading to cellular growth cycle arrest and cell necrosis. At these levels, there is likely formation of DNA and protein adducts, resulting in mutation and the inability of repair mechanisms to recover damaged DNA (Kozekov et al., 2010) followed by apoptosis and DNA fragmentation. Intermediate levels of acrolein showed no evidence of cell necrosis, but still inhibited cell growth, as seen in previous studies (Kehrer and Biswal, 2000; Rahman and MacNee, 2000; Kozekov et al., 2010).

Acrolein toxicity has been linked to two major pathways. It tends to form adducts with the thiol groups of glutathione (GSH), thus depleting cellular GSH and causing antioxidant/oxidant imbalance. GSH depletion leads to activation of apoptosis, most likely through an alteration of the activity of NF- κ B (Kehrer and Biswal, 2000). Kehrer and Biswal further argued that oxidative imbalance is a result of changes in GSH status rather than the formation of oxidation products themselves. Exogenous antioxidants likely alter cellular states not through blockade of ROS but through attenuation of GSH imbalance. Indirect depletion of GSH could also result from ROS generated by damage to the mitochondrial electron transport chain (Luo and Shi, 2005).

A reduction in the efficacy of superoxide dismutase (SOD) seems also to play a major role in oxidant imbalance (Cinci et al., 2010). The transcription factor Nrf2, which is responsible for SOD and GSH generation might be bound or damaged by acrolein, leading to reduction of the antioxidant generation pathway. The importance of Nrf2 in mast

cell inflammatory processes was shown by Rangasamy et al. (2005).

Mast cells degranulate, in part, due to a sudden shift in cytosolic calcium levels (Strider et al., 2011), as is seen in IgE crosslinking. Unlike IgE crosslinking, which induces influx of extracellular calcium, calcium flux induced by acrolein was more likely because of release of intracellular stores of calcium. Although a precise mechanism has not been determined, acrolein may directly or indirectly induce calcium influx via activation of PKC/JNK (Misonou et al., 2005) and/or NF- κ B (Woods et al., 1999).

Taken together, those previous studies corroborate the combined effects we have shown in our experiments. Acrolein exposure leads to both mast cell toxicity at higher concentrations and mast cell activation of intracellular calcium-dependent degranulation pathways resulting most likely from oxidant imbalance. Mast cell derived histamine, leukotrienes, and cytokines are key components of allergic inflammation and asthma (Holgate, 2000; Metz et al., 2007), and thus, our results support the implication of acrolein in the promotion asthma and allergic disease.

The authors wish to thank the following for their technical assistance: Suichiro Endo, MD, Shelby Schultz, and Maren Yngve, and the following individuals for their expert review and guidance: Bill Ameredes, PhD, Mary Moslen, PhD.

REFERENCES

- Arif AA, Delclos GL. 2012. Association between cleaning-related chemicals and work-related asthma and asthma symptoms among healthcare professionals. *Occup Environ Med* 69:35–40.
- Borchers MT, Wert SE, Leikauf GD. 1998. Acrolein-induced MUC5ac expression in rat airways. *Am J Physiol* 274:L573–L581.
- Caldwell JC, Woodruff TJ, Morello-Frosch R, Axelrad DA. 1998. Application of health information to hazardous air pollutants modeled in EPA's cumulative exposure project. *Toxicol Ind Health* 14:429–454.
- Cinci L, Masini E, Bencini A, Valtancoli B, Mastroianni R, Calosi L, Bani D. 2010. Suppression of allergen-induced respiratory dysfunction and airway inflammation in sensitized guinea pigs by Mn(II)(Me(2)DO2A), a novel superoxide scavenger compound. *Free Radic Biol Med* 48:1525–1534.
- Collaco CR, Hochman DJ, Goldblum RM, Brooks EG. 2006. Effect of sodium sulfite on mast cell degranulation and oxidant stress. *Ann Allergy Asthma Immunol* 96:550–556.
- Feng Z, Hu W, Hu Y, Tang MS. 2006. Acrolein is a major cigarette-related lung cancer agent: Preferential binding at p53 mutational hotspots and inhibition of DNA repair. *Proc Natl Acad Sci U S A* 103:15404–15409.
- Fireman P. 2003. Understanding asthma pathophysiology. *Allergy Asthma Proc* 24:79–83.
- Ghilarducci DP, Tjeerdema RS. 1995. Fate and effects of acrolein. *Rev Environ Contam Toxicol* 144:95–146.

- Hargreave FE, Nair P. 2009. The definition and diagnosis of asthma. *Clin Exp Allergy* 39:1652–1658.
- Holgate ST. 2000. The role of mast cells and basophils in inflammation. *Clin Exp Allergy* 30 (Suppl 1):28–32.
- Howard PH. 89 A.D. Acrolein. In: *Handbook of Environmental Fate and Exposure Data for Organic Chemicals*, Vol. 1. Boca Raton, FL: Lewis Publishers, pp. 5–12.
- Kehrer JP, Biswal SS. 2000. The molecular effects of acrolein. *Toxicol Sci* 57:6–15.
- Kozekov ID, Turesky RJ, Alas GR, Harris CM, Harris TM, Rizzo CJ. 2010. Formation of deoxyguanosine cross-links from calf thymus DNA treated with acrolein and 4-hydroxy-2-nonenal. *Chem Res Toxicol* 23:1701–1713.
- Lee RJ, Oliver JM. 1995. Roles for Ca^{2+} stores release and two Ca^{2+} influx pathways in the Fc epsilon R1-activated Ca^{2+} responses of RBL-2H3 mast cells. *Mol Biol Cell* 6:825–839.
- Leikauf GD. 2002. Hazardous air pollutants and asthma. *Environ Health Perspect* 110 (Suppl 4):505–526.
- Leikauf GD, Douplik CA, Leming LM, Wey HE. 1989. Sulfido-peptide leukotrienes mediate acrolein-induced bronchial hyper-responsiveness. *J Appl Physiol* 66:1838–1845.
- Li N, Hao M, Phalen RF, Hinds WC, Nel AE. 2003. Particulate air pollutants and asthma. A paradigm for the role of oxidative stress in PM-induced adverse health effects. *Clin Immunol* 109:250–265.
- Liu XY, Zhu MX, Xie JP. 2010. Mutagenicity of acrolein and acrolein-induced DNA adducts. *Toxicol Mech Methods* 20:36–44.
- Luo J, Shi R. 2005. Acrolein induces oxidative stress in brain mitochondria. *Neurochem. Int.* 46:243–252.
- Matzinger P. 1991. The JAM test. A simple assay for DNA fragmentation and cell death. *J Immunol Methods* 145:185–192.
- McGwin G, Lienert J, Kennedy JJ. 2010. Formaldehyde exposure and asthma in children: A systematic review. *Environ Health Perspect* 118:313–317.
- Metz M, Grimbaldston MA, Nakae S, Piliponsky AM, Tsai M, Galli SJ. 2007. Mast cells in the promotion and limitation of chronic inflammation. *Immunol Rev* 217:304–328.
- Misonou Y, Takahashi M, Park YS, Asahi M, Miyamoto Y, Sakiyama H, Cheng X, Taniguchi N. 2005. Acrolein induces Hsp72 via both PKCdelta/JNK and calcium signaling pathways in human umbilical vein endothelial cells. *Free Radic Res* 39:507–512.
- Myou S, Fujimura M, Nishi K, Ohka T, Matsuda T. 1993. Aerosolized acetaldehyde induces histamine-mediated bronchoconstriction in asthmatics. *Am Rev Respir Dis* 148:940–943.
- Oka T, Sato K, Hori M, Ozaki H, Karaki H. 2002. FcepsilonRI cross-linking-induced actin assembly mediates calcium signaling in RBL-2H3 mast cells. *Br J Pharmacol* 136:837–846.
- Rahman I, MacNee W. 2000. Regulation of redox glutathione levels and gene transcription in lung inflammation: Therapeutic approaches. *Free Radic Biol Med* 28:1405–1420.
- Ranganna K, Yousefipour Z, Nasif R, Yatsu FM, Milton SG, Hayes BE. 2002. Acrolein activates mitogen-activated protein kinase signal transduction pathways in rat vascular smooth muscle cells. *Mol Cell Biochem* 240:83–98.
- Rangasamy T, Guo J, Mitzner WA, Roman J, Singh A, Fryer AD, Yamamoto M, Kensler TW, Tudor RM, Georas SN, Biswal S. 2005. Disruption of Nrf2 enhances susceptibility to severe airway inflammation and asthma in mice. *The Journal of Experimental Medicine* 202:47–59.
- Rashid A, Sadroddiny E, Ye HT, Vratimos A, Sabban S, Carey E, Helm B. 2012. Review: Diagnostic and therapeutic applications of rat basophilic leukemia cells. *Mol Immunol* 52:224–228.
- Roux E, Hyvelin JM, Savineau JP, Marthan R. 1999. Human isolated airway contraction: interaction between air pollutants and passive sensitization. *Am J Respir Crit Care Med* 160:439–445.
- Samoli E, Nastos PT, Paliatatos AG, Katsouyanni K, Priftis KN. 2011. Acute effects of air pollution on pediatric asthma exacerbation: Evidence of association and effect modification. *Environ Res* 111:418–424.
- Shaughnessy RJ, Mcdaniels TJ, Weschler CJ. 2001. Indoor chemistry: Ozone and volatile organic compounds found in tobacco smoke. *Environ Sci Technol* 35:2758–2764.
- Strider JW, Masterson CG, Durham PL. 2011. Treatment of mast cells with carbon dioxide suppresses degranulation via a novel mechanism involving repression of increased intracellular calcium levels. *Allergy* 66:341–350.
- Tam BN, Neumann CM. 2004. A human health assessment of hazardous air pollutants in Portland, OR. *J Environ Manage* 73:131–145.
- Theoharides TC, Kempuraj D, Tagen M, Conti P, Kalogeromitros D. 2007. Differential release of mast cell mediators and the pathogenesis of inflammation. *Immunol Rev* 217:65–78.
- Wang HT, Hu Y, Tong D, Huang J, Gu L, Wu XR, Chung FL, Li GM, Tang MS. 2012. Effect of carcinogenic acrolein on DNA repair and mutagenic susceptibility. *J Biol Chem* 287:12379–12386.
- Woodruff TJ, Axelrad DA, Caldwell J, Morello-Frosch R, Rosenbaum A. 1998. Public health implications of 1990 air toxics concentrations across the United States. *Environ Health Perspect* 106:245–251.
- Woods JS, Ellis ME, Dieguez-Acuna FJ, Corral J. 1999. Activation of NF-kappaB in normal rat kidney epithelial (NRK52E) cells is mediated via a redox-insensitive, calcium-dependent pathway. *Toxicol Appl Pharmacol* 154:219–227.
- Zhang S, Balbo S, Wang M, Hecht SS. 2011. Analysis of acrolein-derived 1,N2-propanodeoxyguanosine adducts in human leukocyte DNA from smokers and nonsmokers. *Chem Res Toxicol* 24:119–124.