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Trimellitic anhydride (TMA) dust induces airway obstruction and eosinophilia in non-sensitized guinea pigs

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Abstract

Trimellitic anhydride (TMA) causes asthma after a latency period of sensitization. In non-sensitized humans and animals, limited studies indicate that TMA exposure may also cause symptoms of asthma without a latency period. Our previous studies (*J. Pharmacol. Exp. Ther.* 296 (2001) 284) in a guinea pig model of TMA-induced asthma demonstrated that sensitization and the complement system were required for eosinophilia. TMA conjugated to guinea pig serum albumin (TMA–GPSA) was used to elicit the response. Since occupational exposure to TMA occurs by inhalation of dust, the present studies determined if exposure to TMA dust in a non-sensitized guinea pig elicited airway obstruction and inflammation, and whether a significantly greater response occurred after a latency period of sensitization. Guinea pigs were intradermally injected with either corn oil (non-sensitized animals) or 30% TMA (sensitized animals). Three weeks later they were challenged by intratracheal insufflation with 1 mg TMA dust or lactose dust (control) using a dry powder delivery device. Pulmonary resistance, dynamic lung compliance, mean arterial blood pressure and heart rate were monitored for 10 min. In non-sensitized guinea pigs, significant increases in pulmonary resistance and decreases in dynamic lung compliance and blood pressure occurred after TMA challenge. In sensitized animals, the same dose of TMA caused significantly greater effects compared to non-sensitized animals. In a separate experiment, cellular infiltration into the lung was determined 24 h after challenge with TMA dust or lactose dust. In both non-sensitized and sensitized animals, eosinophils in the lung tissue were increased after TMA dust challenge compared to controls. Thus, these studies suggest that the response in non-sensitized animals differs depending on whether TMA dust or TMA–GPSA is used to elicit the response. TMA dust elicits significant airway obstruction and eosinophilia in a non-sensitized animal, with even greater airway obstruction occurring in a sensitized animal.

Keywords

Trimellitic anhydride; TMA; Occupational asthma; Bronchoconstriction; Eosinophil; Guinea pig

1. Introduction

Trimellitic anhydride (TMA) is a low molecular weight reactive compound used in paint, epoxies and plastics. It is also an occupational allergen, with exposure typically occurring by inhalation of TMA dust. TMA causes respiratory sensitization and several well defined immunologically-mediated respiratory syndromes, most notably asthma (Grammer et al., 1997; Zeiss et al., 1977). The respiratory effects of TMA in non-sensitized individuals are not

well characterized. However, rhinorrhea, epistaxis, cough, dyspnea and occasional wheezing have been reported to occur upon the first large exposure to TMA in individuals with no evidence of sensitization (Zeiss et al., 1977). Additionally, Grammer et al. (1998) reported a case of one worker at a TMA manufacturing facility that had TMA-induced asthma in the absence of significant IgE, the antibody implicated in the pathogenesis of TMA-induced asthma. Phthalic anhydride, chemically similar to TMA, has been suggested to cause asthma in workers without evidence of sensitization. Popa et al. (1969) described two individuals with phthalic anhydride work-related asthma having undetectable levels of precipitating antibodies and negative skin tests to phthalic anhydride. Wernfors et al. (1986) described three workers with phthalic anhydride related asthma that resolved despite continued exposure, and suggested that temporary heavy exposure may have been the cause. Thus, while occupational exposure to TMA for weeks to years is thought to be required before TMA elicits asthma (Grammer et al., 1997), evidence in humans indicates that TMA and similar compounds also cause asthma without a latency period of sensitization.

Studies using sensitized guinea pigs in which a response is elicited by either inhalation of TMA dust or by intratracheal instillation of TMA conjugated to the protein guinea pig serum albumin (TMA-GPSA) demonstrated a TMA-induced inflammatory response in the lung with marked eosinophilia (Hayes et al., 1992a; Fraser et al., 1995). Few studies have rigorously examined the cell infiltration elicited by either TMA-GPSA or TMA dust in non-sensitized animals. In our recent study using TMA-GPSA, both non-sensitized and sensitized guinea pigs were challenged intratracheally with TMA-GPSA (Larsen et al., 2001). Results demonstrated that sensitization and the complement system were required for lung eosinophilia following TMA-GPSA challenge. In studies using TMA dust to elicit the allergic response, Obata et al. (1992), and Hayes et al. (1992a) used non-sensitized guinea pigs as controls and found that TMA dust challenge resulted in only a few eosinophils in the lung.

TMA-GPSA and TMA dust also cause bronchoconstriction in sensitized guinea pigs (Hayes et al., 1992b; Obata et al., 1992). However, few studies have examined the ability of either TMA-GPSA or TMA dust to elicit bronchoconstriction in a non-sensitized animal. Obata et al. (1992) measured pulmonary resistance in non-sensitized guinea pigs challenged by inhalation with TMA dust. Relative to pre-challenge values, the observed increase in resistance following challenge was not significant. However, the response in non-sensitized animals was not compared to a control. Schaper and Brost (1991) using non-sensitized mice studied the respiratory frequency and patterns of respiratory cycle timing after challenge with aerosols of TMA in acetone. Because the TMA-induced changes in respiratory cycle timing were similar to those caused by the known bronchoconstrictors histamine and acetylcholine, Schaper and Brost (1991) suggested that changes induced by TMA aerosol in non-sensitized mice were indicative of airway constriction. Arts et al. (2001) challenged non-sensitized rats with aerosols of TMA in acetone and noted changes in respiratory frequency and pattern that were similar to those observed by Schaper and Brost (1991) in mice. Neither study in non-sensitized mice nor rats measured changes in pulmonary resistance or lung compliance after TMA challenge.

Acute respiratory effects caused by TMA have not been well defined (Zeiss et al., 1999). Our previous study using non-sensitized guinea pigs challenged with TMA-GPSA demonstrated a requirement for sensitization in TMA-induced eosinophilia (Larsen et al., 2001). However, because occupational exposure to TMA typically occurs by inhalation of the dust, the present studies were designed to determine the importance of sensitization in animals challenged with TMA dust rather than TMA-GPSA. We hypothesize that acute inhalation of TMA dust, such as might occur after an accidental spill, causes airflow obstruction and inflammation in the absence of sensitization, and more pronounced effects in sensitized individuals. Thus, using both nonsensitized and sensitized guinea pigs, TMA dust or the control lactose dust was insufflated into the airways using a dry powder delivery device, and the effects on pulmonary

resistance, dynamic lung compliance, blood pressure, heart rate and infiltration of inflammatory cells into the lung determined.

2. Materials and methods

All animal studies were approved by the University of Minnesota Institutional Animal Care and Use Committee and were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

2.1. Resistance, compliance, blood pressure and heart rate measurements

Female Hartley guinea pigs ($n = 28$, Charles River Laboratories, Portage, MI) weighing 311 ± 4 g (mean $\pm$ 1 SE) were given 100 μ l intradermal injections of either corn oil (non-sensitized animals) or 30% TMA (w/v in corn oil, sensitized animals) on days 1, 3 and 5. Three weeks after sensitization, guinea pigs were anesthetized with ketamine (30 mg/kg im) and xylazine (5 mg/kg im), tracheotomized, a carotid artery cannulated for blood pressure measurements and a jugular vein cannulated for iv injections. Animals were mechanically ventilated at a volume of 10 ml/kg and frequency of 50 cycles/min. Tracheal airflow, transpulmonary pressure, mean arterial blood pressure and heart rate measurements were logged every 10 s using BioSystem XA software (Buxco Electronics, Sharon, CT) as we have previously described (Regal and Klos, 1999). Tracheal airflow and transpulmonary pressure were used to calculate pulmonary resistance and dynamic lung compliance (Amdur and Mead, 1958). Animals were allowed to stabilize for 15–20 min before challenge with either TMA dust or lactose dust. The mean of measurements for 1 min immediately prior to challenge was recorded as the control value. Approximately 1 mg of either TMA (Aldrich Chemical Co.; 97% purity) or lactose (Sigma Chemical Co.) was insufflated into the airways, via the tracheal cannula, just distal to the carina. TMA and lactose were administered as a bolus using a dry powder delivery device (DP-3 Insufflator, PennCentury, Philadelphia, PA; see Concessio et al., 1999; Suarez et al., 2001 for examples of previous use in the guinea pig). The dose delivered was determined by the difference in weight between the loaded delivery device and the weight of the device after insufflation.

2.1.1. Statistical analyses for resistance, compliance, blood pressure and heart rate—Four groups of guinea pigs were considered: non-sensitized, TMA challenged ($n = 8$); non-sensitized, lactose challenged ($n = 4$); sensitized, TMA challenged ($n = 12$); sensitized, lactose challenged ($n = 4$). The Kruskal-Wallis test was used to determine if guinea pig weight, control values of resistance, compliance, blood pressure and heart rate, and the instilled challenge dose differed between the treatment groups. For the time course of the response for individual animals, at each 10 s interval, the ratio of the measurement to the control value was calculated and log transformed to equalize variances prior to statistical analysis. Differences between the treatment groups in the time course were determined by one-tailed t -tests using Satterthwaites' approximation to account for unequal variances (Snedecor and Cochran, 1980). Comparisons made were: non-sensitized, TMA-challenged vs. non-sensitized, lactose-challenged; non-sensitized, TMA-challenged vs. sensitized, TMA-challenged; sensitized, TMA-challenged vs. sensitized, lactose-challenged; non-sensitized, lactose-challenged vs. sensitized, lactose-challenged. Statistically similar results were obtained using data expressed as percent change from control. Statistical analyses were done using JMP software (SAS Institute Inc., Cary NC) and significance was defined as $P < 0.05$. Figures show the mean $\pm$ 1 SE of the percent change from control.

2.2. Cellular infiltration into the lung

An additional group of female Hartley guinea pigs ($n = 25$, Charles River Laboratories, Portage, MI) was used for measuring cell infiltration into the lung 24 h after challenge with either TMA

or lactose. Guinea pigs weighing 292 ± 4 g (mean $\pm$ 1 SE) were given 100 μ l intradermal injections of either corn oil (non-sensitized animals) or 30% TMA (sensitized animals) on days 1, 3 and 5. Cell infiltration in response to TMA challenge was elicited 3 weeks after sensitization. Because antigen-induced bronchoconstriction can cause up to 50% mortality in guinea pigs, animals in this study were injected ip with 6.1 mg/kg of the H1 antagonist pyrilamine 30 min prior to challenge. Guinea pigs were anesthetized with ketamine (24 mg/kg im) and xylazine (1 mg/kg im) and intratracheally challenged with approximately 1 mg of either TMA dust or lactose dust using a dry powder delivery device (DP-3 Insufflator, Penn-Century, Philadelphia, PA). The insufflator was inserted into the trachea such that the orifice was midway between the epiglottis and carina. Our previous studies have shown that peak cell infiltration in response to TMA conjugated to guinea pig serum albumin (TMA-GPSA) occurs 24 h after challenge (Larsen et al., 2001). Therefore, 24 h after challenge with either TMA or lactose, guinea pigs were anesthetized with 75 mg pentobarbital, the lungs were lavaged with six 5 ml aliquots of phosphate buffered saline (PBS) and the lungs were removed for analysis. The bronchoalveolar lavage fluid (BAL) was centrifuged to sediment the cells and the BAL cell pellet was resuspended in 1.0 ml PBS. In the BAL supernatant, the total protein content was determined by the Lowry method (Lowry et al., 1951) and the concentration of C3a was determined by Western blot as we have previously described (Larsen et al., 2001). C3a was used as an indicator of complement system activation. Total white blood cells in BAL were counted by standard methods in a hemacytometer. Differential counts were done on cytopspin preparations of BAL cells (3×10^4 cells) stained with a modified Wright's stain (Diff Quik, American Scientific Products, McGraw Park, IL). Two-hundred cells were counted and identified as eosinophils, neutrophils, or macrophages. Lung lobes were processed as previously described for the measurement of eosinophil peroxidase (EPO) and myeloperoxidase (MPO) activity as an estimate of the number of eosinophils and neutrophils, respectively (Fraser et al., 1995). Red blood cells (RBC) in the cell pellet were lysed by freeze-thawing and the absorbance of hemoglobin (OD₄₁₂) determined as an indicator of lung injury (Fraser et al., 1995).

2.2.1. Statistical analyses for cell infiltration into the lungs—Four groups of guinea pigs were considered: non-sensitized, TMA challenged ($n = 8$); non-sensitized, lactose challenged ($n = 4$); sensitized, TMA challenged ($n = 9$); sensitized, lactose challenged ($n = 4$). The Kruskal-Wallis test was used to determine if guinea pig weight and the instilled challenge dose differed between the treatment groups. The effects of sensitization (non-sensitized or sensitized), challenge (TMA or lactose) and interactions of sensitization and challenge on numbers of cells in the lung were determined by two-way ANOVA modeling unequal variances with a log linear variance model. Two groups for variance were used; animals challenged with TMA dust and those challenged with lactose. Data were log transformed prior to ANOVA. Statistical analyses were done using JMP software (SAS Institute Inc., Cary NC) and significance was defined as $P < 0.05$. Figures show the geometric mean $\pm$ 1 SE.

3. Results

3.1. Resistance, compliance, blood pressure and heart rate

Guinea pig weights at the time of challenge were not significantly different between treatment groups (457 ± 8 g). Control values for pulmonary resistance (0.35 ± 0.01 cm H₂O/ml/s), dynamic lung compliance (0.29 ± 0.01 ml/cm H₂O), mean arterial blood pressure (38 ± 1 mm Hg) and heart rate (230 ± 3 beats/min) were not different between the treatment groups. The mean challenge dose was 1.2 ± 0.1 mg dust and did not significantly differ between treatment groups.

In non-sensitized animals challenged with TMA, pulmonary resistance increased (Fig. 1) and dynamic compliance decreased (Fig. 2) immediately after challenge, whereas changes were significantly less in lactose-challenged animals. In non-sensitized animals, changes in resistance and compliance were significantly greater in TMA- vs. lactose-challenged animals indicating that TMA-induced bronchoconstriction occurred in the absence of sensitization. However, TMA-induced changes in resistance and compliance in non-sensitized animals were significantly smaller than those measured in sensitized animals challenged with TMA. In sensitized animals, challenge with TMA caused greater changes in resistance and compliance than did lactose. During limited portions of the time course, sensitized guinea pigs challenged with lactose exhibited greater changes in resistance and compliance than did non-sensitized animals challenged with lactose.

Blood pressure in all treatment groups generally decreased throughout the experiments (Fig. 3). The momentary initial increase in blood pressure is associated with the lack of ventilation during the intratracheal insufflation procedure. In sensitized TMA-challenged animals, a triphasic blood pressure response was observed in which an initial decrease in blood pressure was followed by a relatively large but transient increase, then a final decrease. This pattern of change in blood pressure was not observed in non-sensitized animals challenged with TMA, or in lactose-challenged control animals. In non-sensitized animals after either TMA- or lactose-challenge, blood pressure decreased similarly. Comparing the TMA-challenged groups, sensitized guinea pigs had a significantly greater decrease in blood pressure than did non-sensitized animals during the last several minutes of the monitoring period. In sensitized guinea pigs, TMA challenge resulted in a significantly greater initial decrease in blood pressure as well as a greater sustained decrease in blood pressure than in lactose-challenged animals.

Heart rate increased approximately 5% after TMA challenge in both non-sensitized and sensitized animals, while in lactose challenged animals there was no change (data not shown). Approximately 2.5 min after TMA challenge, heart rate in sensitized animals began declining and at 10 min after challenge had decreased by 35%. Decreases in heart rate were not observed in the other groups.

3.2. Cellular infiltration

Guinea pig weights at the time of challenge were not significantly different between treatment groups (430 ± 6 g). The mean challenge dose was 1.4 ± 0.2 mg dust and did not significantly differ between treatment groups.

Numbers of inflammatory cells in the BAL 24 h after TMA challenge tended to increase in sensitized animals but not in non-sensitized animals (Fig. 4). Two-way ANOVA did not detect significant sensitization, challenge or sensitization-challenge interaction effects with respect to numbers of inflammatory cells in the BAL fluid. In the lung tissue (Fig. 5), a significant challenge effect for EPO activity was detected by two-way ANOVA indicating that EPO activity was significantly greater after TMA challenge than after lactose challenge. Significant sensitization and/or challenge effects for MPO activity in the lung tissue were not detected.

Numbers of red blood cells (RBC) in the BAL increased after TMA challenge in sensitized but not in non-sensitized guinea pigs (Fig. 6). Two-way ANOVA detected a significant sensitization-challenge interaction effect. Additional analysis using one-tailed single-degree of freedom contrasts indicated that for sensitized guinea pigs, numbers of RBC were significantly greater in the TMA- than in the lactose-challenged animals. Non-sensitized animals challenged with either TMA or lactose were not different. Total protein in the BAL did not vary with either sensitization and/or challenge as determined by two-way ANOVA. The concentration of C3a in the BAL was slightly elevated in non-sensitized compared to

sensitized animals, and two-way ANOVA detected a sensitization effect (data not shown). However, neither a challenge effect nor a sensitization/challenge interaction effect was detected for C3a.

Since the effects of TMA dust may resolve within 24 h of challenge, a separate group of animals was examined 6 h after challenge with either TMA dust or lactose dust to determine if differences in inflammatory cells, RBC, total protein and C3a occurred. Significant TMA-induced increases were not detected at 6 h (data not shown).

4. Discussion

Clinical studies by Zeiss et al. (1977) described non-sensitized workers having TMA-induced symptoms of wheeze and dyspnea. In addition, studies monitoring respiratory patterns in non-sensitized mice and rats have suggested TMA causes airway obstruction (Schaper and Brost, 1991; Arts et al., 2001). However, in non-sensitized guinea pigs following inhalation of TMA dust, pulmonary resistance did not significantly increase relative to pre-challenge values (Obata et al., 1992). The response in non-sensitized animals was not compared to a control. Our present study is the first to show that TMA dust causes airway obstruction in non-sensitized animals. This was demonstrated by significant increases in pulmonary resistance and decreases in dynamic lung compliance following TMA challenge. Additionally, increases in pulmonary resistance and decreases in dynamic lung compliance after TMA challenge were significantly greater in sensitized than in non-sensitized animals at the same challenge dose of TMA. This study is also the first to report significant TMA-induced eosinophilia in the lung of non-sensitized animals.

TMA dust and TMA-GPSA have both been used to elicit a response in animal models of occupational asthma. Previous studies by others using sensitized guinea pigs have demonstrated that TMA dust and TMA-GPSA cause similar inflammatory and bronchoconstrictor effects (Fraser et al., 1995; Hayes et al., 1992a,b; Obata et al., 1992). However, in non-sensitized guinea pigs, the effects of TMA dust compared to TMA-GPSA differ. In this study using TMA dust, eosinophils in non-sensitized as well as sensitized animals increased after challenge. Additionally, there was no significant activation of the complement system in either non-sensitized or sensitized animals. In contrast to the current study, our previous study using TMA-GPSA demonstrated no increase in eosinophils in non-sensitized animals, but significant complement system activation in both non-sensitized and sensitized animals (Larsen et al., 2001). TMA-GPSA-induced bronchoconstriction in non-sensitized guinea pigs has not been rigorously assessed. Differences between the two studies suggest use of a TMA-protein conjugate to elicit the response may not be a realistic model of occupational asthma caused by TMA in which exposure is typically via inhalation of dust.

As noted above, differences in TMA-induced eosinophilia were observed between the current study using TMA dust and our previous study using TMA-GPSA (Larsen et al., 2001). These differences were not likely due to variations in the time course of the response since 6 h after challenge neither TMA dust in this study, nor TMA-GPSA conjugate in our previous study caused changes in inflammatory cells. The tendency for increased neutrophils and macrophages in sensitized but not in non-sensitized guinea pigs following TMA dust challenge in this study is consistent with our previous study using TMA-GPSA (Larsen et al., 2001). However, in the previous study, the TMA-GPSA induced changes in neutrophils and macrophages in sensitized animals were statistically significant.

TMA exposure in this study simulated a single acute accidental exposure as opposed to an extended low level occupational exposure. The target dose of 1 mg TMA dust (~2.2 mg/kg) used to elicit the asthmatic response is comparable to exposures in previous studies of others

examining the respiratory effects of TMA in sensitized guinea pigs. These previous studies challenged animals by inhalation to concentrations of TMA dust ranging from 8–150 mg/m³ for 15–60 min (Botham et al., 1989; Hayes et al., 1992a, 1993; Obata et al., 1992; Tao et al., 1991). Assuming an average range of respiration rate and tidal volume (Terril and Clemons, 1998), we estimated guinea pigs in these studies were exposed to 0.004–7.7 mg TMA. During an inadvertent spill, a person would receive a similar exposure to TMA as used in the present study by inhaling TMA at 150 mg/m³ for 30 min (assuming an average adult of 70 kg weight with a ventilation rate of 35 l/min incurred during moderate work; EPA 1997). Short term exposure to TMA at concentrations of ~ 150 mg/m³ during an accident are conceivable since levels of TMA dust in a factory during routine operation have been reported to exceed 20 mg/m³ (van Tongeren et al., 1995).

Pulmonary resistance in non-sensitized guinea pigs in this study increased approximately 70% after TMA challenge. This is similar to the approximate 50% increase in pulmonary resistance in non-sensitized guinea pigs shown in data presented by Obata et al. (1992). However, in Obata's study, pulmonary resistance in non-sensitized animals after challenge was not statistically different from pre-challenge values. Guinea pigs in Obata's study were challenged with 150 mg/m³ TMA dust for 30 min, and were exposed to an estimated 0.25–1.5 mg TMA, assuming an average range of respiration rate and tidal volume (Terril and Clemons, 1998). In Obata's study, the measurements of pulmonary resistance were highly variable and a control dust was not used for comparison. The authors concluded that TMA-induced bronchoconstriction did not occur in non-sensitized guinea pigs.

Mean arterial blood pressure decreased after TMA challenge in non-sensitized as well as sensitized guinea pigs in this study. In sensitized, but not in non-sensitized animals, TMA dust elicited a pronounced tri-phasic change in blood pressure; an initial decrease followed by a transient return towards pre-challenge blood pressure levels before a final decrease. A similar pattern of change in blood pressure was previously observed in sensitized guinea pigs after iv injection of ovalbumin (Regal et al., 1993). Arakawa et al. (1993), eliciting a response in guinea pigs with TMA–GPSA, also observed a decrease in blood pressure in both non-sensitized and sensitized animals. Additionally, the decrease in blood pressure in sensitized animals was significantly greater than that in non-sensitized animals. Hayes et al. (1992a) by contrast did not detect significant changes in blood pressure in either non-sensitized or sensitized guinea pigs after inhalation of TMA dust (12 mg/m³ for 30 min). However, because measurements were made only at 2, 8 and 24 h after challenge, immediate changes in blood pressure would have been missed.

Lung injury as determined by RBC in the BAL fluid was apparent only in sensitized animals. These results are consistent with work done by Leach, Zeiss and coworkers in rats that indicated the development of hemorrhagic lung foci in response to TMA dust was immunologically mediated (Leach et al., 1988; Zeiss et al., 1988). The present study is also consistent with our previous study showing increased numbers of RBC in the BAL fluid in sensitized but not in non-sensitized guinea pigs after TMA–GPSA challenge (Larsen et al., 2001). Hemoptysis in humans has been reported to occur in sensitized individuals after TMA exposure (Zeiss et al., 1977), and TMA-induced lung injury observed in sensitized animals is consistent with immunologically mediated hemoptysis in humans.

Mechanisms causing TMA-induced symptoms of asthma in non-sensitized animals are not known. The airway obstruction and influx of eosinophils observed in this study may have been due to TMA-induced release of allergic mediators or neural reflexes triggered by TMA. The neural reflexes could potentially be triggered by either allergic mediators, acid in the lung formed by the hydrolysis of TMA, or by TMA dust itself. Sensory irritation (stimulation of trigeminal nerve endings in the eyes, nose and throat) was not a factor since TMA was

administered intratracheally. However, a neural reflex caused by stimulation of nerve endings in the respiratory tract (pulmonary irritation) may be involved. Peptides released by stimulation of neurons in the respiratory tract can cause bronchoconstriction and inflammation (Ricciardolo, 2001). Schaper and Brost (1991) using non-sensitized mice, and similarly Arts et al. (2001) using non-sensitized rats, suggested that observed changes in respiratory cycle timing following TMA challenge were indicative of reflex bronchoconstriction in the lower respiratory tract. However, Arts et al. (2001) noted that TMA was not like typical pulmonary irritants in that there was a rapid onset of response, increased respiratory frequency at low TMA concentrations, rapid recovery following exposure, no change in tidal volume and no change in lung weights due to edema. Regardless of the mechanism, this study demonstrates that TMA dust causes airway obstruction and eosinophilia in the absence of a latent period of sensitization.

The effects of acute inhalation of TMA or other acid anhydrides in non-sensitized individuals are not well documented. Since the response of non-sensitized animals to TMA-GPSA and TMA dust differ, the present study suggests that using TMA-protein conjugates to elicit a response may not be an appropriate model of asthma where occupational exposure occurs via inhalation of TMA dust. In addition, the results of the present study demonstrate that TMA dust causes the airway obstruction and eosinophilia of asthma without prior sensitization, with even greater airway obstruction occurring at the same challenge dose after sensitization.

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Fig. 1.

Percent change in pulmonary resistance after challenge with either TMA dust or lactose dust at time 0. Data represent the mean $\pm$ 1 SE for 4–12 animals in each treatment group. SE bars are shown only for selected values to increase clarity of the plot. Solid horizontal lines below the x-axis indicate periods of significant difference ($*P < 0.05$) for the given comparisons.

Fig. 2.

Percent change in dynamic lung compliance after challenge with either TMA dust or lactose dust at time 0. Data represent the mean $\pm$ 1 SE for 4–12 animals in each treatment group. SE bars are shown only for selected values to increase clarity of the plot. Solid horizontal lines below the x -axis indicate periods of significant difference ($*P < 0.05$) for the given comparisons.

Fig. 3. Percent change in mean arterial blood pressure after challenge with either TMA dust or lactose dust at time 0. Data represent the mean $\pm$ 1 SE for 4–12 animals in each treatment group. SE bars are shown only for selected values to increase clarity of the plot. Solid horizontal lines below the x -axis indicate periods of significant difference ($*P < 0.05$) for the given comparisons.

Fig. 4. Numbers of eosinophils, neutrophils and macrophages in the BAL 24 h after challenge with either TMA dust or lactose dust. Data represent geometric means ± 1 SE for 4–9 animals in each treatment group. Significant effects ($P < 0.05$) were not detected.

Fig. 5.

EPO and MPO activity in lung tissue 24 h after challenge with either TMA dust or lactose dust. EPO and MPO activity are used as indicators of the numbers of eosinophils and neutrophils, respectively. Data represent geometric means $\pm$ 1 SE for 4–9 animals in each treatment group. *Significant ($P < 0.05$) challenge effect as indicated by two-way ANOVA.

Fig. 6.

Numbers of RBC and total protein in the BAL 24 h after challenge with either TMA dust or lactose dust. Data represent geometric means $\pm$ 1 SE for 4–9 animals in each treatment group.

†Significant ($P < 0.05$) difference between TMA-sensitized animals challenged with lactose and those challenged with TMA as indicated by one-tailed single degree of freedom contrast.