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RESEARCH ARTICLE

Pre-exposure to sulfur dioxide attenuates most allergic reactions upon trimellitic anhydride challenge in sensitized Brown Norway rats

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Abstract

Irritant-induced inflammation of the airways may aggravate respiratory allergy induced by chemical respiratory allergens. Therefore, it was studied whether airway irritation by sulfur dioxide (SO₂) would enhance respiratory allergic reactions to trimellitic anhydride (TMA), using a rat model. Brown Norway (BN) rats were topically sensitized, subsequently exposed for a single time or repeatedly to 300 ppm SO₂, and challenged by inhalation to a distinctly irritating or minimally irritating concentration of TMA after the (last) SO₂ exposure. Repeated exposure to SO₂ alone reduced breathing frequency during exposure, and caused epithelial alterations including hyperplasia and squamous metaplasia, and infiltration of polymorphonuclear inflammatory cells into nasal tissues, larynx, trachea, and bronchi/bronchioli. Histopathological changes were less prominent after 1 day of SO₂ exposure. Repeated pre-exposure to SO₂ reduced the number of TMA-induced apnoeas, in an SO₂ exposure duration-dependent manner. This effect of SO₂ on TMA-induced functional allergic reactions (apnoeas) was distinct only when the TMA challenge concentration was not too irritating itself. Repeated pre-exposure to SO₂ reduced TMA-induced laryngeal ulceration, goblet-cell hyperplasia, and inflammation in the lungs in most animals, regardless of the TMA challenge concentration. The SO₂-induced replacement of normal respiratory epithelium by less sensitive, squamous epithelium may offer an explanation for the, unexpected, reduced allergic manifestation. However in a few animals, SO₂ appeared to facilitate TMA-induced irritation, probably due to incomplete protection. Overall, SO₂ exposure of TMA-sensitized rats reduced TMA-related allergic respiratory responses in most animals.

Keywords: Brown Norway rats; respiratory allergy; respiratory irritation; sulfur dioxide; trimellitic anhydride

Introduction

Occupational exposure to low-molecular-weight (LMW) compounds such as diisocyanates, acid anhydrides, and certain reactive dyes can cause sensitization of the respiratory tract, resulting in allergic inflammation and pulmonary hypersensitivity reactions upon re-exposure to the same compound (Kimber et al., 1996). In addition to allergens, humans can be exposed to respiratory irritants like SO₂, NO₂, certain aldehydes, fluorides, and chlorides, especially in workplaces. The latter compounds are not allergenic, i.e., these are active without prior exposure but can cause allergy-like symptoms such as respiratory-tract inflammation and bronchoconstriction at high concentrations (Baur et al., 1998; Chan-Yeung and Malo, 1995; Medina-Ramon

et al., 2005; Samet, 1995). Epidemiological studies have demonstrated an association between elevated levels of irritants in air and exacerbation of asthmatic symptoms. In these studies it has been suggested that respiratory irritation may increase not only the incidence of allergic reactions when also exposed to an allergen, but also their severity and duration. Moreover, it may change the type of allergy of allergic reactions (Rusznak et al., 1996; Strand et al., 1997; 1998; Venables and Chan-Yeung, 1997).

This was confirmed in studies in guinea pigs and Brown Norway rats in which exposure to SO₂ or NO₂ during sensitization with the high-molecular-weight (HMW) compound ovalbumin (OVA) increased the allergic airway responses during OVA challenge (Park et al., 2001; Riedel et al., 1988;

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Siegel et al., 1997). The underlying mechanism may be the induction of aspecific airway inflammation, which increases epithelial permeability (D'Amato et al., 2000). Other studies in experimental animals have shown enhancement of the allergic reaction to HMW protein allergens when the animals were co-exposed to irritants such as acrolein, acetic acid, or NO₂ during the challenge phase (Gilmour, 1995; Morris et al., 2003).

As epidemiological studies have demonstrated an association between elevated concentrations of irritants in air and exacerbation of asthmatic symptoms, we examined in this study in rats the effect of pre-exposure to an airway irritant, SO₂, on the elicitation of airway hypersensitivity reactions by the allergen trimellitic anhydride (TMA), i.e., during the challenge phase. In contrast to the HMW allergens used in the abovementioned studies, TMA is a typical respiratory LMW allergen and is known to have induced various allergic respiratory diseases (Zeiss et al., 1992). TMA is also known for its airway-irritating effects in humans. Although TMA also can evoke respiratory irritant reactions at high concentrations, in rats these reactions could easily be distinguished from allergic reactions (Arts et al., 2004). Inhalation of SO₂ is known to induce bronchoconstrictive reactions in both healthy and asthmatic adults, in children, and in animals (Atzory et al., 1992; Dodge et al., 1985). It is considered to cause upper-respiratory-tract irritation by forming sulfuric acid upon contact with water (Lipsett, 1992). It does so at relatively low concentrations (ca. 5–50 ppm; Alarie et al., 1975; Hirsch et al., 1975; Lewis et al., 1973; Lipsett, 1992; Min et al., 1994; Scanlon et al., 1987). At high concentrations (viz. 200–250 ppm), SO₂ has also induced effects in the lower respiratory tract of rats (Kodavanti et al., 2000; Long et al., 1999).

This study examined whether respiratory exposure of TMA-sensitized BN rats to SO₂ before inhalation challenge with TMA, affected the allergic or irritant reaction to TMA, as judged by breathing parameters, bronchoalveolar lavage, and histopathology. The concentration of SO₂ used was based on a preceding range-finding study; the concentrations of TMA were based on previous studies (Arts et al., 2001, 2004). We examined the difference between a single and repeated exposure to SO₂, and the difference between a minimally and distinctly irritating concentration of TMA. In addition, other TMA-sensitized and -challenged BN rats were exposed to SO₂ before a second TMA challenge; the latter group may resemble allergic individuals.

Material and methods

Animals and maintenance

Female, 7- to 8-wk-old, inbred Brown Norway (BN) rats were purchased from Charles River Deutschland GmbH (Sulzfeld, Germany). The animals were acclimatized for at least 5 days before the start of the study; they were 9–10 wk old at the start of the study. They were kept under conventional laboratory conditions, 3–4 animals per cage, at 22 ± 3°C and at 30–70% relative humidity. The light cycle was 12 h light/12 h dark.

They received the standard powder diet (RM3, SQC, Special Diet Service, Witham, UK), and unfluoridated tap water ad libitum. All animal procedures were approved by the TNO Commission of Animal Welfare.

Materials

Trimellitic anhydride (TMA; purity 97%) was obtained from Aldrich (Brussels, Belgium), sulfur dioxide (SO₂; 1 ppm = 2.7 mg/m³; purity 99.98%) from Hoek Loos BV (Dieren, The Netherlands), acetone from Merck (Darmstadt, Germany), and raffinated olive oil from Sigma Diagnostics, Inc., St. Louis, MO.

Study design

Concentration-range finding test with SO₂: Groups of 5 female BN rats were exposed to either 150 or 300 ppm SO₂ during 2.5 h or 5 h per day, 5 days/wk for 2 wk to establish the exposure concentration and duration at which inflammatory changes could be seen at the bronchio/bronchioli level. Body and organ (liver, kidney, and lung) weights and histopathological results were compared to unexposed control animals, and breathing pattern was compared to pre-exposure data. A concentration of 300 ppm and a duration of 2.5 h per day was chosen for further studies.

TMA sensitization, pre-exposure to SO₂ and TMA challenge at a distinctly irritating concentration: Groups of 6 female BN rats were exposed to 300 ppm SO₂ 2.5 h/day for 12 days after TMA sensitization but prior to TMA challenge (see Table 1). A distinctly irritating TMA challenge concentration of 30–40 mg/m³ was used based on previous studies (Arts et al., 2001, 2004); the interval between the last SO₂ exposure and TMA challenge was 24 h.

TMA sensitization, pre-exposure to SO₂ and TMA challenge at a minimally irritating TMA challenge concentration: Groups of 4–6 female BN rats were exposed to 300 ppm SO₂ 2.5 h/day for 1 or 10 days after TMA sensitization but prior to TMA challenge (see Table 1). A minimally irritating TMA challenge concentration of 20 mg/m³ was chosen (Arts et al., 2001, 2004); the interval between (the last) SO₂ exposure and TMA challenge was 1 h.

TMA sensitization, TMA challenge, pre-exposure to SO₂, and a second TMA challenge at a minimally irritating TMA challenge concentration: Groups of 4–6 female BN rats were exposed to 300 ppm SO₂ 2.5 h/day for 10 days after TMA sensitization but prior to TMA challenge, and/or exposed to 300 ppm SO₂ 2.5 h/day for 1 day after the first TMA challenge but prior to the second TMA challenge (see Table 1). A minimally irritating TMA challenge concentration of 20 mg/m³ was chosen (Arts et al., 2001, 2004); the interval between (the last) SO₂ exposure and TMA challenge was 1 h.

Sensitization and challenge procedure

Animals received 150 µl TMA (50% w/v) in a 4:1 (v/v) mixture of acetone and olive oil (AOO) as the vehicle on each flank (approximately 12 cm² each), which had been shaved with an electric razor at least 2–3 days earlier. Seven days

Table 1. Exposure schemes used.

Purpose of the study	Group	TMA sensitization	First SO ₂ exposure period(days)	First TMA challenge (day)	Second SO ₂ exposure(day)	Second TMA challenge(day)	Necropsy(day)
Concentration-range finding test with SO ₂	—	—	Days 0-13 (10 days)	—	—	—	14
TMA sensitization, pre-exposure to SO ₂ , and TMA challenge at a distinctly irritating TMA challenge concentration	-/+ +/+	— Days 0 and 7	— —	30 ## 30 ##	— —	— —	31 31
	-/SO ₂ (12)/+ +/SO ₂ (12)/+	— Days 0 and 7	8-29 (12 days) 8-29 (12 days)	30 ## 30 ##	— —	— —	31 31
TMA sensitization, pre-exposure to SO ₂ , and TMA challenge at a minimally irritating TMA challenge concentration	-/SO ₂ (10)/+ -/SO ₂ (1)/+ +/ SO ₂ (10)+ +/ SO ₂ (1)/+	— — Days 0 and 7 Days 0 and 7	7-21 (10 days) 21 (1 day) 7-21 (10 days) 21 (1 day)	21 # 21 # 21 # 21 #	— — — —	— — — —	22 22 22 22
TMA sensitization, TMA challenge, pre-exposure to SO ₂ , and a second TMA challenge at a minimally irritating TMA challenge concentration	+ / SO ₂ (10)/+ / SO ₂ (1)/+ + / + / + + / + / SO ₂ (1) + / + / SO ₂ (1) / +	Days 0 and 7 Days 0 and 7 Days 0 and 7 Days 0 and 7	10-23 (10 days) — — —	23 # 21 # 21 # 21 #	29 — 28 28	29 # 29 # — 28 #	30 30 29 29

Note. Control groups were dermally treated with vehicle or exposed to clean air instead of SO₂; see for sensitization concentrations the Material and Methods section. In the range-finding test, SO₂ concentrations were 150 or 300 ppm, and exposure duration was 2.5 or 5 h/day. In all other experiments, SO₂ concentration was 300 ppm, exposure duration was 2.5 h/day. Mean challenge concentrations of TMA were 20 mg/m³ (#) or 30–40 mg/m³ (##). In the group labels, the first - or + indicates no and yes TMA sensitization, respectively, and the second and/or third + indicates TMA challenge; the indication of SO₂ followed by a number indicates the number of days of exposure to SO₂.

after the first sensitization, they received 75 μ l TMA (25% w/v) on the dorsum of each of both ears. Controls received vehicle (AOO). The dermal sensitization route was chosen because of its effectivity as proven in several respiratory sensitization and allergic disease models with LMW compounds (reviewed by Arts and Kuper, 2007). Animals were challenged by inhalation to TMA for 7 min. A few additional groups were re-challenged with TMA (Table 1).

Exposure of the animals, test atmosphere generation, and analysis

During SO₂ exposure, the rats were individually restrained in Battelle tubes and each tube was then placed into inhalation units (ADG Developments Ltd. Codicote, Herts, UK) for head/nose-only exposure to the test atmosphere. The unit had a volume of approximately 50 L. During SO₂ exposure, metered amounts of SO₂ (measured with a rotameter) were diluted with dry pressurized air, and subsequently diluted with mass-flow controlled amounts of humidified air. The resulting test atmospheres were passed to the inhalation units. During periods of non-exposure, all tubing was flushed with nitrogen to prevent corrosion. SO₂ concentrations were measured by infrared analysis using a photoacoustic infrared absorption analyzer (dual gas monitor, type 3426, Bruell & Kjaer, Naerum, Denmark) operated at 8.8 μm. At 8.8 μm interference from water vapour is not present. The response of the dual gas monitor was calibrated using sample bags filled with known amounts of SO₂ gas. Calibration curves with coefficients of determination of 0.999 were obtained. The mean exposure air flows were 27 L/min, i.e., at least 1 L/min per animal. Daily mean temperature of the test atmospheres was between 21.0 and 23.1°C, and daily mean relative humidity was between

51 and 57%. For measurement of changes in respiration (breathing frequency and pattern) during SO₂ exposure, a part of the test atmosphere was passed to a second unit consisting of a central chamber onto which four whole-body plethysmographs were connected and in which four rats were placed (Arts et al., 1998).

For respiratory challenge to TMA, rats were treated as described previously (Arts et al., 1998). Using this experimental setup, 2 TMA-sensitized and 2 vehicle-treated rats at a time, were first exposed to fresh air for 23 min (pre-challenge period) and then to the TMA atmosphere for exactly 7 min (challenge period), followed by a recovery period of 23–30 min. An all-glass nebulizer designed at the Institute TNO Quality of Life, was used to generate the test atmospheres from freshly prepared solutions of TMA in acetone (Schaper and Brost, 1991). The acetone concentration was kept between 3000 and 5000 ppm ($\sim 7\text{--}12\text{ g/m}^3$), levels that are considered to be far below the level inducing sensory irritation (Alarie, 1973; De Ceaurriz et al., 1981; Schaper and Brost, 1991) and that indeed did not induce changes in breathing pattern in either sensitized or non-sensitized rats (Arts et al., 1998, 2001). The challenge concentrations of TMA were based on a previous study performed in BN rats (Arts et al., 2004). Atmospheric concentrations of TMA were determined gravimetrically by filter sampling. The particle size distribution of TMA in the test atmospheres was determined using a 10-stage cascade impactor (Andersen, Atlanta, GA). During inhalation challenge, due to the small sampling airflow rate and the large total volume required for analytical and particle size determinations, samples were not collected during the challenge exposure but immediately prior to or after challenge. The mean mass median aerodynamic diameter (MMAD) of

the aerosolized TMA particles was between 0.9 and 1.2 μm with geometric standard deviations (gsd) between 2.5 and 2.9. The exposure air flows were on average 19 L/min.

Respiratory measurements

To obtain an indication of the airway irritant properties of SO_2 , respiration (breathing frequency, tidal volume, and breathing pattern) was semi-continuously monitored by means of recording the pressure signal before and during several days of SO_2 exposure at 300 ppm (maximally 4 rats could be measured at a time during SO_2 exposure).

Respiration was monitored prior to, during, and after TMA challenge in all animals. Prior to TMA challenge, the respiration was monitored approximately 20 s each min starting 6 min prior to the actual challenge. During the 7-min challenge period, respiration was monitored continuously, whereas after challenge, respiration was monitored approximately 20 s each min for the first 3–10 min and then at 5-min intervals for a total period of approximately 23–30 min. Finally, respiration was monitored at least 5 times within a 10-min period after a 20-min acclimatisation period 24 h after exposure. Therefore, mean values were obtained from 6 measurements before challenge, from 12–14 measurements during challenge, from 6–7 measurements immediately after challenge, and from 5–6 measurements 24 h after challenge.

Local effects, body and organ weights, necropsy, and serum IgE levels

All animals were observed at least once daily and weighed shortly before the first treatment, at weekly intervals thereafter, and just prior to necropsy. At necropsy, animals were anaesthetised with nembutal, killed by exsanguination from the abdominal aorta, and examined grossly for abnormalities. Lungs with trachea and larynx were removed and weighed to investigate local toxicity; liver, kidneys, and spleen were removed and weighed to examine systemic toxic effects. Individual serum samples were prepared from blood withdrawn prior to sensitization via the orbital plexus and via the abdominal aorta at necropsy. The serum samples were stored at -20°C until analysis of total serum immunoglobulin (Ig) E levels by means of an enzyme-linked immunosorbent assay (ELISA) as described earlier (Arts et al., 1997). Two animals with high pre-bleed values (higher than mean value $\pm 2\text{SD}$) were left out for statistical analysis.

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) measurements were performed in all groups. At necropsy, the lungs of the animals were lavaged according to Hooftman et al. (1988). This was performed 2 times with a volume of 40 ml saline/kg body weight in case the whole lung was lavaged, or 2 times with a volume of 23 ml saline/kg body weight in case the right lung lobes (accessory lung lobes) were lavaged, after binding of the left (main) lobe, which was used for histopathological evaluation. The total amount of retracted lavage fluid was weighed and retained on ice. The bronchoalveolar cells were isolated from the supernatant by centrifugation

($250\times g$) during 5 min at 4°C and resuspended in 0.5 ml saline to assess total cell and differential cell numbers. Total cell numbers were counted using an automated haematology analyzer (K-800, Sysmex, Toa, Kobe, Japan). The percentage of viable cells was determined using an acridine orange/ethidium bromide staining method in combination with fluorescence microscopic evaluation. For differential cell counts, cytopspins were prepared and stained with May-Grunwald-Giemsa. At least 200 cells were counted per animal to determine absolute numbers and percentages of macrophages/ monocytes, lymphocytes, neutrophils and eosinophils. Supernatants were used for determination of total protein, lactate dehydrogenase (LDH), alkaline phosphatase (ALP), γ -glutamyltransferase (GGT), and *N*-acetyl glucosaminidase (NAG), using an automatic analyzer (Hitachi 911, Hitachi Instruments Division, Japan).

Airway histopathology

Neutral, phosphate-buffered 4% (v/v) formaldehyde was used to inflate and preserve the complete lungs or left lung lobe of the animals, and to preserve the nasal tissues, larynx, and trachea. Next these organs were embedded in paraffin wax. Cross sections of the nasal tissues were prepared at six standardized levels (Woutersen et al., 1994). The larynx was sectioned longitudinally including the epiglottis and the ventral pouch (three sections). The anterior part of the trachea was sectioned longitudinally (three sections). Lung lobes were cut at one sagittal level. Sections (5 μm) were stained with haematoxylin and eosin and examined microscopically. In animals of the pre-study (exposure to SO_2 only) the lower respiratory tract (trachea and lungs) was examined; in the other animals the complete respiratory tract was investigated.

Statistics

Body weights were analysed by one-way analysis of covariance (COVAR) followed by the two-sided Dunnett's multiple comparison test. Total serum IgE concentrations (after logarithmic transformation), organ weights, absolute cell numbers and biochemical parameters in BAL fluid were determined by analysis of variance (ANOVA)–Dunnett's test. Relative cell numbers in BAL fluid were analysed by Kruskal–Wallis non-parametric analysis of variance followed by the Mann–Whitney *U*-test. The respiratory parameters recorded before, during, and after TMA challenge at a distinctly irritating concentration were first analysed by two-way ANOVA with sensitization and SO_2 exposure as covariates. Because statistical differences were observed amongst the four groups, but no interaction, non-sensitized groups were combined and compared to sensitized groups regardless of SO_2 exposure. Next, the differences in challenge/pre-challenge and post-challenge/pre-challenge values between test and control groups were evaluated using repeated-measures ANOVA using Greenhouse Geisser correction followed by post hoc contrast tests. The latter statistical analyses were also used in the study with a minimally irritating challenge concentration of TMA.

Results

Effect of SO₂

Exposure of rats to 300 ppm SO₂ for 5 h/day reduced respiratory frequency to about 60% of the initial mean value, but the respiratory frequency returned to about 90% of the initial value just prior to the next exposure. The decreases in breathing frequency were accompanied by increases in tidal volume. Exposure of rats to 300 ppm SO₂ for 5 h/day also clearly reduced body weight gain. No changes in body weight gain were observed in the other groups. Changes in organ weights (liver, kidneys, and lungs) were absent. In addition, SO₂ induced concentration- and exposure-duration-related degenerative and hyperplastic changes in the epithelium lining the trachea, main bronchi, and bronchioli. The changes were severe at 300 ppm of SO₂ for 5 h/day and mild to moderate at 300 ppm for 2.5 h/day or 150 ppm during 5 h/day. Exposure at 150 ppm during 2.5 h induced minor effects in 1 animal only (data not shown). The concentration and duration of SO₂ selected to study its effect on allergic reactions to TMA were chosen to be mildly irritating and was 300 ppm for 2.5 h/day.

Effect of SO₂ on respiratory allergy to TMA

Local effects, body and organ weights, and serum IgE levels

Flank application of TMA caused local dermal erythema and encrustations; ear application of TMA caused no grossly visible local reactions. Skin reactions to TMA generally appeared 1 day after application and persisted for a few days. A single inhalation exposure to TMA did not change the weights of body, kidney, and liver, but it increased absolute and relative lung weights in sensitized animals regardless of SO₂ exposure and regardless of TMA challenge concentration (data not shown).

Mean total serum IgE levels were significantly increased in TMA-sensitized rats (ca. 1200–3000 ng/ml) regardless of SO₂ exposure and regardless of TMA challenge concentration, compared to IgE levels in non-sensitized rats (ca. 250–600 ng/ml).

Changes in breathing frequency

As in the pre-study with SO₂, respiratory frequency decreased during SO₂ exposure at 300 ppm to about 60% of the initial mean value, but returned to about 90% of the initial value just prior to the next exposure (data not shown). The decreases in breathing frequency were accompanied by increases in tidal volume.

Regardless of SO₂ exposure, TMA challenge at a distinctly irritating concentration induced in sensitized rats a statistically significant decrease in breathing frequency during challenge, followed by a statistically significant increase 24 h after challenge; the time-response curve was actually parabolic (J-shaped; Figure 1, see also Figure 2). Sensitized and non-sensitized groups were combined because analysis of breathing frequency revealed a significant effect of TMA challenge of sensitized rats and a significant effect of

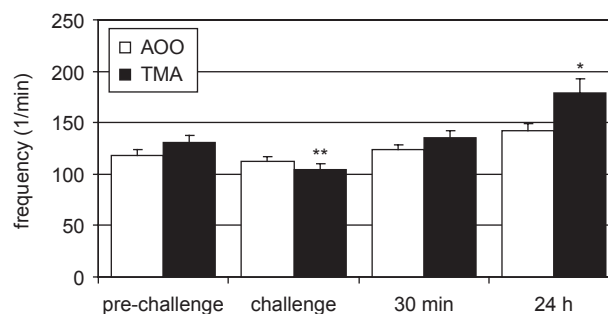


Figure 1. Changes in breathing frequency in animals following TMA sensitization, exposure to SO₂, and inhalation challenge to a distinctly irritating concentration of 30–40 mg/m³ TMA. SO₂ concentration was 300 ppm, and exposure duration was 2.5 h/day (12 days exposure). Mean breathing frequency of BN rats was measured before, during, and after challenge to TMA. Statistics: repeated measures ANOVA/post hoc contrast test; **p* < .05; ***p* < .01. Because statistical significant differences were observed amongst the four groups (–/+ , +/+ , –/SO₂/+ , and +/SO₂/+) but no interaction, non-sensitized groups were combined (–/+ and –/SO₂/+) and compared to sensitized groups (+/+ and +/SO₂/+) regardless of SO₂ exposure. Results are expressed as mean ± SEM. Number of animals: 6 rats/group. Statistics: repeated-measures ANOVA/post hoc contrast test; * *p* < .05; ***p* < .01.

SO₂ exposure, but no interaction; i.e., functional changes induced by TMA challenge in sensitized rats were not influenced by pre-exposure to SO₂.

Also in animals challenged at a minimally irritating TMA concentration, the time-response curves in breathing frequency induced by TMA sensitization and challenge were parabolic and followed a second-order polynomial regression. Using this exposure scheme, 10-day pre-exposure to SO₂ resulted in flattening of the J-shaped curve when compared to 1-day pre-exposure, indicating a less stronger decrease in breathing frequency during challenge, and a less strong increase in breathing frequency 24 h later. This difference almost reached statistical significance (Figure 2).

Such a flattening effect of 10-day pre-exposure to SO₂, and to a lesser extent of 1-day pre-exposure to SO₂, was also present during and after the first challenge when compared to TMA-sensitized and -challenged rats that had not been pre-exposed to SO₂ (Figure 3a). This was also true in the case of a 1-day pre-exposure to SO₂ before and after the second challenge (Figure 3b). Statistical significance was reached in both cases.

Changes in breathing pattern

Typical for irritation: A challenge exposure to TMA at a concentration of 30–40 mg/m³ induced pulmonary irritation in both TMA-sensitized and non-sensitized rats, i.e., a spiked instead of a waveform breathing pattern and a slight increase in the pause between breaths at the end of expiration (at peak expiratory pressure; Schaper and Brost, 1991; Arts et al., 2001, 2003). Further, the time of inspiration and expiration appeared to be slightly decreased, indicating that this concentration was functionally irritating to the respiratory tract. Effects of SO₂ pre-exposure on this spiked breathing pattern induced at the challenge concentration of 30–40 mg/m³ TMA were not observed.

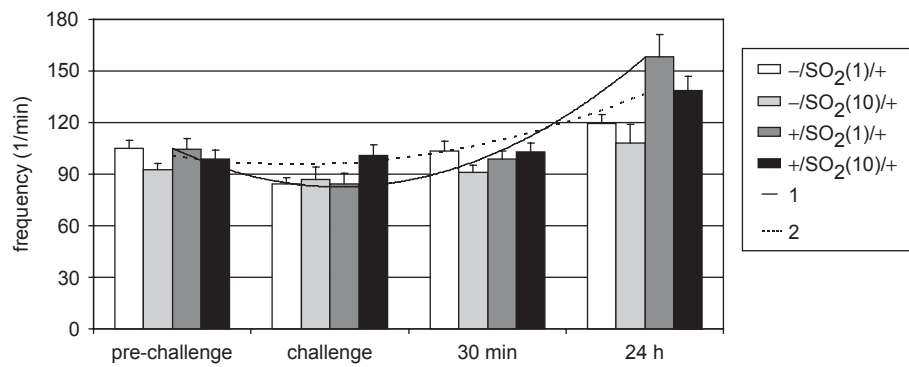


Figure 2. Changes in breathing frequency in animals following TMA sensitization, exposure to SO₂, and inhalation challenge to a minimally irritating concentration of 20 mg/m³ TMA. SO₂ concentration was 300 ppm, and exposure duration was 2.5 h (single exposure) or 2.5 h/day (10 days exposure). Mean breathing frequency of BN rats was measured before, during, and immediately after challenge, and 24 h after challenge to TMA. Results are expressed as mean ± SEM. The lines connecting the different time points were calculated using a second-order polynomial. Line 1 (continuous) connects data of the third group (+/SO₂(1)/+); line 2 (dotted) connects data of the fourth group (+/SO₂(10)/+). Number of animals: 4–6 rats/group. Statistics: repeated-measures ANOVA/post hoc contrast test; the Greenhouse Geisser corrected interaction between group and time between the +/SO₂(10)/+ and the +/SO₂(1)/+ almost reached statistical significance ($p = .078$).

A challenge concentration of 20 mg/m³ TMA did not induce pulmonary irritation, i.e., no spiked breathing pattern was observed. A breathing pattern typical of irritation was, however, occasionally observed during TMA challenge at this concentration in animals pre-exposed to SO₂ (data not shown).

Typical for TMA-induced respiratory allergy: Apnoeic periods, i.e., irregularly lengthened pauses between a varying number of breaths, typical for TMA-induced allergy, were only observed in TMA-sensitized rats during TMA challenge. A single exposure to SO₂ before TMA challenge diminished the incidence (number of animals showing apnoeic breathing) and degree (number of apnoeas per animal). Prolonged pre-exposure to SO₂ before TMA challenge even completely prevented the occurrence of apnoeas (Table 2; Figure 4). The diminishing effect of SO₂ was also present during the second TMA challenge (Table 2).

Bronchoalveolar lavage

Increased levels of lactate dehydrogenase (LDH), *N*-acetylglucosaminidase (NAG), and protein were found in TMA-sensitized rats 24 h after TMA challenge at a distinctly irritating concentration, regardless of SO₂ pre-exposure (Figure 5). Increases in total cell number and eosinophils were observed in sensitized groups also regardless of SO₂ pre-exposure; the numbers of neutrophils and lymphocytes were higher in TMA-sensitized and -challenged animals pre-exposed to SO₂ than in those not pre-exposed to SO₂ (Figure 6).

In all sensitized groups challenged with a minimally irritating TMA concentration, increased protein, LDH, and NAG contents were generally also found. Statistical significance was reached in protein levels in most groups, whereas for LDH and NAG statistical significance was reached only incidentally. Highest levels were generally seen in sensitized animals that had received SO₂ for 1 day compared to those that had received SO₂ for 10 days (Figure 7). Highest numbers of total cells, neutrophils, and eosinophils were also seen in sensitized animals that had received SO₂ for 1

Table 2. Presence of apnoeic periods in breathing pattern during TMA challenge at a minimally irritating concentration.

Group description	Presence of apnoeic periods during first challenge	Presence of apnoeic periods during second challenge
-/SO ₂ (1)/+	—	NA
+ /SO ₂ (1)/+	+	NA
-/SO ₂ (10)/+	—	NA
+ /SO ₂ (10)/+	—	NA
+ /SO ₂ (10)/+ /SO ₂ (1)/+	—	+
+ /+ /+	++	++
+ /+ /SO ₂ (1)	++	NA
+ /+ /SO ₂ (1)/+	++	+

Note. Number of animals: 4–6 rats/group. Control groups were dermally treated with vehicle or exposed to clean air instead of SO₂; see for sensitization concentrations the Material and Methods section. SO₂ concentration was 300 ppm, and exposure duration was 2.5 h (single exposure) or 2.5 h/day (10 days exposure). Mean challenge concentration of TMA was 20 mg/m³; — = apnoeic breathing pattern not present; + = apnoeic breathing pattern occasionally present (degree) and/or present in a few animals only (incidence); ++ = apnoeic breathing pattern frequently present (degree) and/or present in several animals (incidence); NA = not applicable.

day compared to those that had received SO₂ for 10 days. Statistical significance was only occasionally reached due to a high standard deviation in the control group caused by one outlier (Figure 8).

In TMA-sensitized animals challenged twice, the highest levels of protein, LDH and NAG were generally found in animals without pre-exposure to SO₂, followed by the groups with SO₂ pre-exposure (Figure 7). The highest numbers of total cells and eosinophils were also found in animals without pre-exposure to SO₂, followed by animals receiving SO₂ pre-exposure for 10 days and 1 day, and finally by animals receiving SO₂ pre-exposure for 1 day (Figure 8).

Histopathological airway changes

Nasal passages: Exposure to SO₂ for 1, 10, or 12 days induced epithelial-cell degeneration and hyperplasia, oedema, and

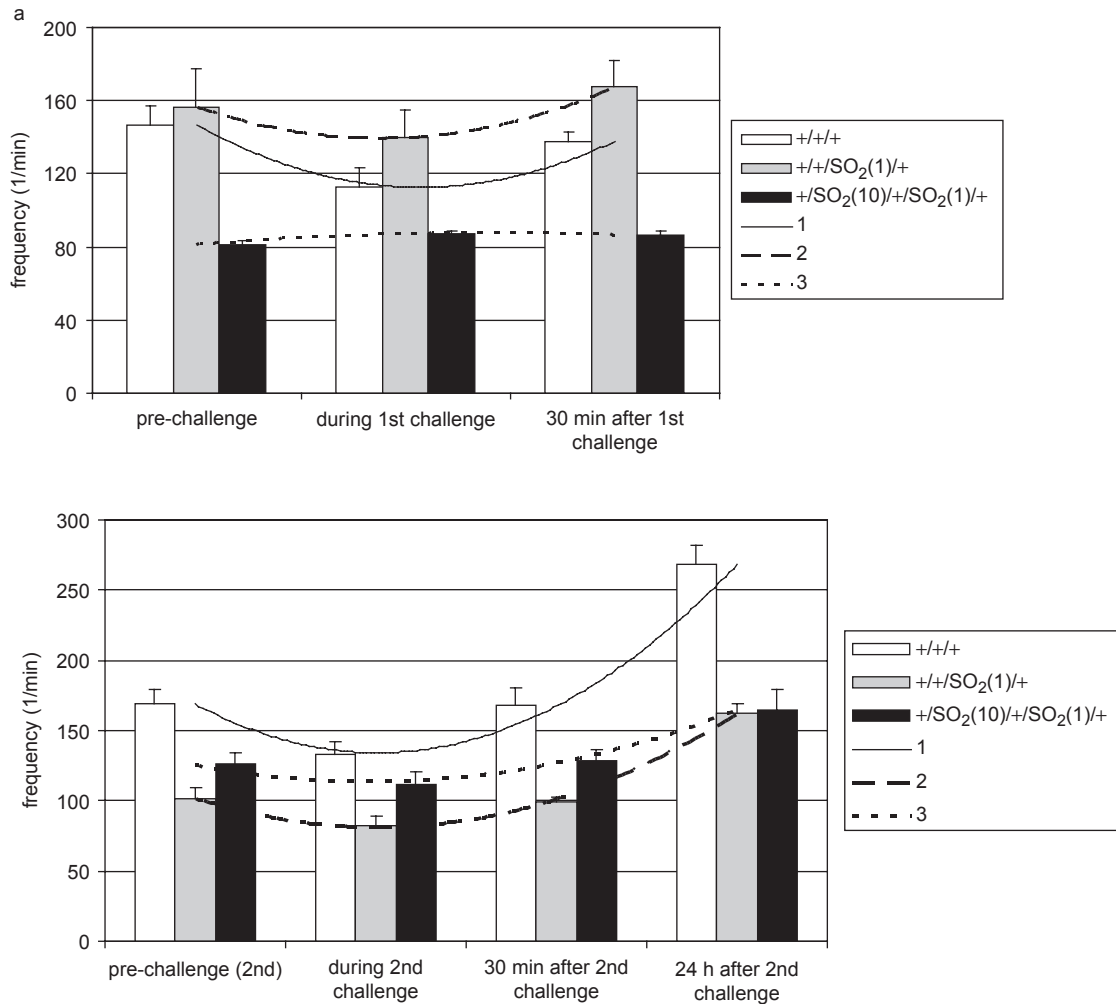


Figure 3. (a) Changes in breathing frequency in animals following TMA sensitization, exposure to SO₂, and inhalation challenge to a minimally irritating concentration of 20 mg/m³ TMA. SO₂ concentration was 300 ppm, exposure duration was 2.5 h (single exposure) or 2.5 h/day (10 days exposure). Mean breathing frequency of BN rats was measured before, during, and immediately after the first challenge to TMA. Results are expressed as mean $\pm$ SEM. The lines connecting the different time points were calculated using a second order polynomial. Line 1 (continuous) connects data of the first group (+/+/+); line 2 (striped) connects data of the second group (+/+/SO₂(1)/+); line 3 (dotted) connects data of the third group (+/SO₂(10)/+/SO₂(1)/+). Number of animals: 4–6 rats/group. Statistics: repeated-measures ANOVA/post hoc contrast test; significant group effects ($p < .01$) and interactions between group and time ($p < .01$) were observed between the third group (+/SO₂(10)/+/SO₂(1)/+) and the two other groups. (b) Changes in breathing frequency in animals following TMA sensitization, exposure to SO₂, and inhalation challenge to a minimally irritating concentration of 20 mg/m³ TMA. SO₂ concentration was 300 ppm, exposure duration was 2.5 h (single exposure) or 2.5 h/day (10 days exposure). Mean breathing frequency of BN rats was measured before, during, immediately after, and 24 h after the second challenge to TMA. Results are expressed as mean $\pm$ SEM. The lines connecting the different time points were calculated using a second-order polynomial. See for a description of the lines the legend of Figure 3a. Number of animals: 4–6 rats/group. Statistics: repeated measures ANOVA/post hoc contrast test; significant group effects ($p < .01$) and interactions between group and time ($p < .01$ to $p < .05$) were observed between the first group (+/+/+) group and the two other groups.

granulocytic inflammation along the entire nasal passages. Proximally in the nasal passages effects were more severe in 1-day-exposed animals than in prolonged exposed animals; distally effects were regardless of exposure duration. TMA challenge did not induce histopathological changes in the nasal tissues, regardless of its concentration, nor was it influenced by the SO₂-induced effects in these organs (data not shown).

Larynx: The effects of SO₂ exposure (squamous epithelial metaplasia and hyperplasia) in both sensitized and non-sensitized rats and the effects of the TMA challenge (ulceration and a mixed or granulocytic inflammatory cell infiltrate at the ventral site of the epiglottis) in non-sensitized rats

were considered to be due to irritation of the compounds (summarized under ‘Total score irritation’ in Tables 3 and 4). In TMA-sensitized rats, the TMA challenge-induced ulceration and cell infiltrate was more pronounced, and, moreover, a protruding mixed granulomatous inflammation was induced in a few rats. This type of inflammation is observed solely in sensitized and challenged animals and was therefore summarized under ‘Total score allergy’ in Tables 3 and 4. SO₂ exposure prior to challenge led to a reduction in irritation-related inflammatory cell infiltrate and ulceration (–/+ compared to –/SO₂, and +/+ compared to +/SO₂/+; Table 3), in an SO₂ exposure duration-related manner (12 days SO₂ being most effective, e.g., –/SO₂(12)/+

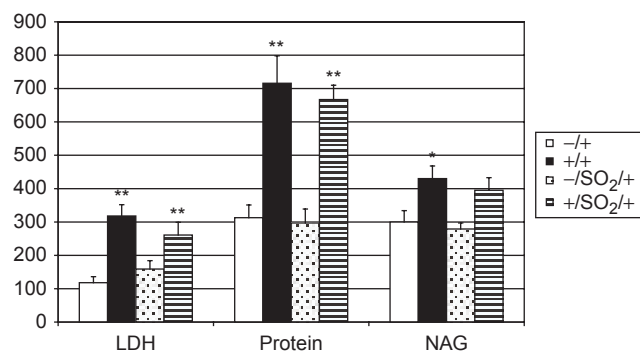


Figure 4. Changes in biochemical parameters in bronchoalveolar lavage fluid at necropsy of animals following TMA sensitization, exposure to SO_2 , and inhalation challenge to a distinctly irritating concentration of 30–40 mg/m^3 TMA. SO_2 concentration was 300 ppm, exposure duration was 2.5 h/day (12 days exposure). LDH = lactate dehydrogenase (U/L); protein (mg/L); NAG = N-acetyl glucosaminidase (U/L $\times 10\text{E}-2$). Results are expressed as mean $\pm$ sem. Number of animals: 6 rats/group. Statistics: ANOVA/Dunnett's test; * $p < .05$, ** $p < .01$ (first mentioned group as control group).

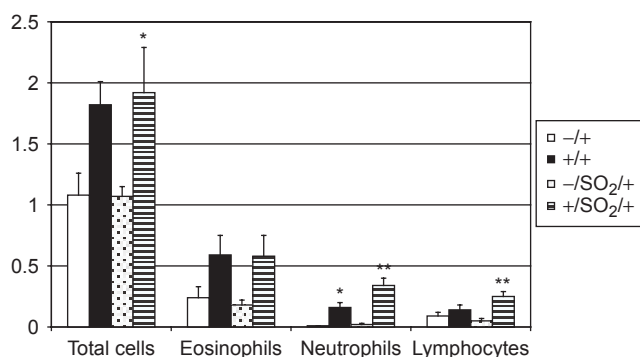


Figure 5. Changes in cellular parameters in bronchoalveolar lavage fluid at necropsy of animals following TMA sensitization, exposure to SO_2 , and inhalation challenge to a distinctly irritating concentration of 30–40 mg/m^3 TMA. SO_2 concentration was 300 ppm, and exposure duration was 2.5 h/day (12 days exposure). Results are expressed as mean $\times 10\text{E}6 \pm$ SEM; total volume is 0.5 ml. Number of animals: 6 rats/group. Statistics: ANOVA/Dunnett's test; * $p < .05$, ** $p < .01$ (first mentioned group as control group).

group in Table 3 compared to $-\text{SO}_2(10)/+$ and $-\text{SO}_2(1)/+$ groups in Table 4). In addition, SO_2 exposure prior to a minimally irritating, but not a distinctly irritating, TMA challenge in TMA-sensitized animals led to a reduction in the allergic protruding inflammation, to such extent that this allergic inflammation was absent in all $+\text{SO}_2(1)/+$ animals and almost all $+\text{SO}_2(10)/+$ animals. Instead, these animals showed a mixed or granulocytic inflammatory cell infiltrate, associated with irritation. Interestingly, in 2 TMA-sensitized and -challenged animals, exposure to SO_2 for 10 days aggravated this inflammation (see footnote under Table 4).

The allergic laryngeal lesions were most severe in the animals challenged twice with TMA without additional exposure to SO_2 . SO_2 preceding TMA challenge diminished the allergy score in the 2 groups and, when given only for 1 day prior to the second TMA challenge, also the irritation score (Table 4).

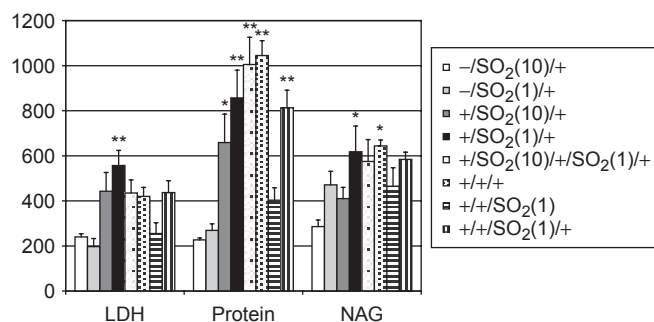


Figure 6. Changes in biochemical parameters in bronchoalveolar lavage fluid at necropsy of animals following TMA sensitization, exposure to SO_2 , and inhalation challenge to a minimally irritating concentration of 20 mg/m^3 TMA. SO_2 concentration was 300 ppm, exposure duration was 2.5 h (single exposure) or 2.5 h/day (10 days exposure). LDH = lactate dehydrogenase (U/L); protein (mg/L); NAG = N-acetyl Glucosaminidase (U/L $\times 10\text{E}-2$). Results are expressed as mean $\pm$ SEM. Number of animals: 4–6 rats/group. Statistics: ANOVA/Dunnett's test; * $p < .05$, ** $p < .01$ (first mentioned group as control group).

Trachea: In the trachea, epithelial hyperplasia was induced following exposure to SO_2 . TMA challenge did not induce histopathological changes in the trachea, regardless of its concentration, nor was it influenced by the SO_2 -induced effects in these organs (data not shown).

Lungs: SO_2 alone induced bronchiolar/bronchial squamous epithelial metaplasia and hyperplasia, decreased the prominent goblet-cell expression as observed in the larger bronchioli of untreated BN rats, and increased the goblet-cell hyperplasia in the smaller bronchioli (data not shown).

Challenge of sensitized rats with a distinctly irritating TMA concentration increased the histopathological changes compared to the challenged, non-sensitized rats (increased irritation score), but did not induce additional histopathology (no allergy score; Table 3). Overall, there were no clear morphological effects of SO_2 on the TMA-induced pulmonary irritation, except for a decrease in goblet cell hyperplasia following 12 days of SO_2 pre-exposure ($-\text{SO}_2(10)/+$ group compared to $+/+$, and $+/+$ group compared to $+\text{SO}_2(10)/+$; Table 3). In TMA-sensitized rats challenged with a minimally irritating TMA concentration pre-exposed to SO_2 for 10 days, the irritation score was comparable to that of 1 day pre-exposure. No additional allergy score was observed (Table 4).

The most distinct pulmonary lesions, namely, severe peribronchiolar inflammation and severe haemorrhages, were observed in TMA-sensitized animals challenged twice with TMA; 1-day pre-exposure to SO_2 reduced the lung inflammation and the severity of the haemorrhages when given prior to the second challenge with TMA. The allergy score was comparable amongst these groups (Table 4).

Discussion

The effect of SO_2 -induced airway irritation and inflammation was studied on the allergic airway reactions elicited by the low-molecular-weight (LMW) allergen TMA. Irritation by TMA itself is mainly characterized by the presence of

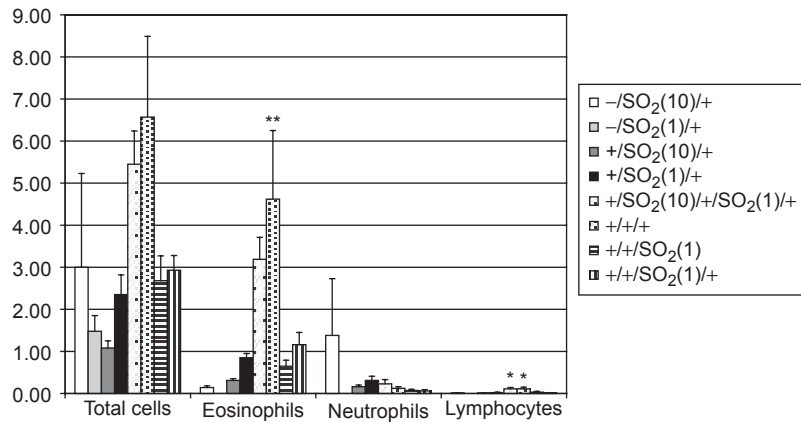


Figure 7. Changes in cellular parameters in bronchoalveolar lavage fluid at necropsy of animals following TMA sensitization, exposure to SO₂, and inhalation challenge to a minimally irritating concentration of 20 mg/m³ TMA. SO₂ concentration was 300 ppm, and exposure duration was 2.5 h (single exposure) or 2.5 h/day (10 days exposure). Results are expressed as mean $\times 10^6 \pm$ SEM; total volume is 0.5 ml. Number of animals: 4–6 rats/group. Statistics: ANOVA/Dunnett's test; * $p < .05$, ** $p < .01$ (first mentioned group as control group).

Table 3. Histopathological changes^a in the larynx and lungs following TMA sensitization, exposure to SO₂ for 12 days, and inhalation challenge to a distinctly irritating concentration of TMA.

Group	-/+	+/+	-/SO ₂ (12)/+	+ /SO ₂ (12)/+
LARYNX				
Squamous metaplasia/hyperplasia	—	—	1	3
Epithelial ulceration	2	3	—	1
Mixed or granulocytic inflammatory cell infiltrate	1	2	—	1
Total score irritation	3	5	1	5
Protruding mixed/granulomatous inflammation	—	2	—	2
Total score allergy	0	2	0	2
LUNGS				
Squamous metaplasia/epithelial hyperplasia	—	—	2	2
Goblet cell hyperplasia main bronchi/bronchioli	1	2	—	—
Inflammation around terminal bronchioli	1	2	1	2
Alveolitis	1	2	1	2
Total score irritation	3	6	4	6
Total score allergy	0	0	0	0

Note. Number of animals: 6 rats/group. Control groups were dermally treated with vehicle or exposed to clean air instead of SO₂; see for sensitization concentrations the Material and Methods section. SO₂ concentration was 300 ppm, and exposure duration was 2.5 h/day (12 day exposure). Mean challenge concentration of TMA was 30–40 mg/m³. Animals were killed and airways were removed for examination 24 h after challenge.

^a Histopathological changes are expressed in a mean score, on a scale of 1 (very slight) to 5 (very severe). Allergic changes are those that are exclusive for the sensitized/challenged groups. The mean scores are summed up to give a total score for irritation or allergy.

a spiked breathing pattern instead of a wave form, with a slight increase in the pause between breaths at the end of expiration (at peak expiratory pressure; Schaper and Brost, 1991; Arts et al., 2001, 2003), and with histopathological changes in the airways (see also Table 5). Sensitization and challenge with TMA induced significantly increased total serum IgE levels and apnoeic breathing pattern and reduced breathing frequency during TMA challenge, and increased relative lung weight and laryngeal and pulmonary inflammation 24 h after challenge, as seen previously. Thus, breathing patterns between sensitized and non-sensitized rats differed to such an extent that sensitization and challenge-induced changes could be clearly distinguished from irritant-induced ones. Also, the differences in the type of histopathological changes following inhalation exposure to TMA in the larynx and lungs of sensitized and non-sensitized rats enabled comparison of allergy-induced and irritation-induced changes (Table 5; Arts et al., 1998, 2003, 2004).

The concentration of 300 ppm SO₂ for 2.5 h/day for 2 weeks was considered optimal because of mild airway irritation in the deeper airways in the absence of effects on body weight and other indications of distress. The irritation consisted of slower and deeper breathing as observed by Long et al. (1999), distinct epithelial alterations, such as squamous metaplasia, and slight to moderate inflammation in the upper and lower respiratory tract. The lower-respiratory-tract effects were in line with those reported by Kodavanti et al. (1999), Long et al. (1999), and Min et al. (1994) using comparable SO₂ concentrations (between 100 to 500 ppm) in rats. Even lower concentrations of SO₂ (ca. 5–50 ppm) mainly caused upper-respiratory-tract irritation in various species (Alarie et al., 1975; Hirsch et al., 1975; Lewis et al., 1973; Lipsett, 1992; Scanlon et al., 1987). In the present study, the histopathological changes observed in the nasal passages and trachea were considered to be solely due to SO₂ exposure, as these tissues were unaffected in TMA-sensitized animals following TMA challenge (Table 5; Arts et al., 1998, 2003, 2004).

Table 4. Histopathological changes^a in the larynx and lungs following TMA sensitization and one or two TMA inhalation challenges at a minimally irritating concentration, with or without exposure to SO₂ for 1 or 10 days.

Group	-/SO ₂ (1)/+	+ /SO ₂ (1)/+	-/SO ₂ (10)/+	+ /SO ₂ (10)/+	+ /SO ₂ (10)/+ / SO ₂ (1)/+	+ /+/+	+ /+ /SO ₂ (1)/+	+ /+ /SO ₂ (1)
LARYNX								
Squamous metaplasia/ hyperplasia	1	2	1	2	1	2	—	—
Epithelial ulceration	1	1	—	—	1	1	—	—
Mixed or granulocytic inflammatory cell infiltrate	1	1	1	1#	1	—	1	—
Total score irritation	3	4	2	3	3	3	1	0
Hyperplastic respiratory epithelium	—	—	—	—	—	—	1	—
Protruding mixed/ granulomatous inflammation	—	—	—	1	1	4	—	2
Total score allergy	0	0	0	1	1	4	1	2
LUNGS								
Squamous metaplasia/ epithelial hyperplasia	—	1	1	1	—	—	—	—
Goblet cell hyperplasia main bronchi/bronchioli	—	1	2	2	1	1	—	2
Inflammation around terminal bronchioli	1	1	1	1	2	2	1	2
Alveolitis	—	1	1	—	1	1	1	1
Haemorrhages	—	—	—	—	2	2	—	2
Total score irritation	1	4	5	4	6	6	2	7
Fibrosis	—	—	—	—	1	1	1	—
Mononuclear cell infiltrate	—	—	—	—	1	—	—	—
Perivascular plasma cells	—	—	—	—	1	1	1	—
Total score allergy	0	0	0	0	3	2	2	0

Note. Number of animals: 4–6 rats/group. Control groups were dermally treated with vehicle or exposed to clean air instead of SO₂; see for sensitization concentrations the Material and Methods section. SO₂ concentration was 300 ppm, and exposure duration was 2.5 h (single exposure) or 2.5 h/day (10 days exposure). Mean challenge concentration of TMA was 20 mg/m³. Animals were killed and airways were removed for examination 24 h after challenge.

^a Histopathological changes are expressed in a mean score, on a scale of 1 (very slight) to 5 (very severe). Allergic changes are those that are exclusive for the sensitized/challenged groups. The mean scores are summed up to give a total score for irritation or allergy. #: in 4/6 TMA-sensitized and -challenged animals, the inflammatory cell infiltrate was reduced when compared to +/+ animals (see Table 3); however, in 2/6 animals the inflammatory cell infiltrate was aggravated.

Interaction of SO₂ (12-day exposure) with TMA-induced functional allergic reactions, as measured by recording the breathing parameters, was not very distinct when the TMA challenge was given at an irritating concentration of 30–40 mg/m³ after the last SO₂ exposure, possibly because the irritation by TMA overshadowed the reactions induced by SO₂. SO₂ exposure increased the numbers of neutrophils and lymphocytes in BAL of TMA-sensitized and -challenged animals.

At a minimally irritating concentration of TMA both the sensitisation-related and irritation-related changes in the breathing parameters and the allergic inflammation in larynx and lungs were reduced by pre-exposure to SO₂. In the larynx, the allergic-type laryngeal inflammation was even replaced by irritant-related inflammation due to pre-exposure to SO₂. Prolonged exposure to SO₂ generally led to more reduction of the allergic reactions than a short-term exposure. The reduction was most pronounced in the larynx, probably because SO₂ has its highest impact on the upper respiratory tract including the larynx. In TMA-sensitized animals challenged

twice with TMA, a short exposure to SO₂ before the second challenge reduced the changes in breathing parameters and the laryngeal allergic inflammation, compared to animals not exposed to SO₂. The interactions between SO₂ and TMA are considered to be due to both the irritating capacity of the allergen itself, reflected here in the different TMA challenge concentrations, and the quantity of the effects by the irritant, here the duration of the pre-exposure to SO₂.

The results of the present study are largely in accordance with another study performed with TMA but with pre-exposure to synthetic amorphous silica (SAS) particles rather than SO₂. Exposure to SAS for 6 days prior to TMA challenge diminished the effect of TMA on tidal volume, laryngeal ulceration, laryngeal inflammation, and the number of BAL (lung) eosinophils in most animals, but aggravated laryngeal squamous metaplasia and inflammation in a single animal (Arts et al., 2008). The results of the present study are also in accordance with a few other experimental studies, using HMW allergens such as ovalbumin. A reduction in ovalbumin (OVA)-induced cellular

Table 5. Summary of effects of irritation, allergy and combined effects.

	Effects of SO ₂	Effects of irritation by TMA(observed in both sensitized (+/+) and non-sensitized (-/+) animals)	Effects of TMA allergy(observed in sensitized animals only)	Effects of SO ₂ on TMA-induced irritation and allergy
Functional changes / breathing pattern	Decreased breathing frequency during exposure to SO ₂	A spiked instead of a waveform breathing pattern and a slight increase in the pause between breaths (TMA concentration-related)	Decreased breathing frequency with apnoeic periods during challenge, increased breathing frequency 24 h after challenge	Less reduced breathing frequency during challenge and less increased breathing frequency 24 h after challenge; lower numbers or even absence of apnoeas(SO ₂ exposure duration-related)
Lung weight	No effects	No effects ^a	Increased absolute and relative lung weight 24 h after challenge	No combined effects
Bronchoalveolar lavage (cellular)	Not examined	No effects ^a	Increased numbers of total cells and eosinophils	Increased numbers of neutrophils and lymphocytes; lower increases in cell differentials following repeated SO ₂ exposures
Bronchoalveolar lavage (biochemical)	Not examined	No effects ^a	Increased levels of LDH, NAG and protein	Lower levels following repeated SO ₂ exposures
Total serum IgE	Not examined	No effects ^a	Increased total serum IgE	No combined effects
Histopathology nose	Epithelial cell degeneration and hyperplasia, oedema and granulocytic inflammation	No effects ^a	No effects	No combined effects
Histopathology larynx	Squamous metaplasia en hyperplasia(SO ₂ exposure duration-related)	Ulceration and inflammatory cell infiltrate (at 20 and 30-40 mg/m ³ TMA); more pronounced in +/+ animals	Granulomatous inflammation	Reduction of irritation-related cell infiltrate and ulceration*; reduction more pronounced at 20 mg/m ³ TMA; reduction of allergic inflammation of animals challenged twice
Histopathology trachea	Epithelial hyperplasia	No effects ^a	No effects	No combined effects
Histopathology lungs	Squamous metaplasia and hyperplasia in bronchi/bronchioli	Goblet cell hyperplasia and inflammation(at 20 and 30-40 mg/m ³ TMA) more pronounced in +/+ animals	No effects	Reduction of goblet cell hyperplasia at 30-40 mg/m ³ and reduction of lung irritation following a second challenge with 20 mg/m ³ TMA

*With the exception of two animals in which TMA-induced irritation was increased.

^a Based on previous studies in which non-sensitized/non-challenged (-/-) control groups were used (Arts et al., 1998, 2001, 2003).

pulmonary inflammation was observed in OVA-sensitized mice exposed to 0.7 or 5 ppm NO₂; these mice had been exposed to NO₂ for 2 h each day for 3 days following each day's OVA aerosol challenge (Hubbard et al., 2002). Another study showed decreases in OVA-induced bronchoconstriction and in eosinophil and neutrophil numbers in BAL in OVA-sensitized guinea pigs following pre-exposure for 4 wk to 0.5 ppm benzaldehyde but not to 0.2 ppm acetaldehyde (Lacroix et al., 2002). The results of one epidemiological study were suggestive as well for a reduction in allergic symptoms by irritation: Symptoms of allergy were less frequent in East Germany than in West German children, while the former were exposed to higher air pollution levels (SO₂ and dust) than the latter (Oppermann and Kramer, 1999). Further studies are needed to unravel the complex interactions between airway irritation and respiratory allergy. In that respect, it should be noted that protein allergens are generally not as irritating to the airways as chemical allergens.

Our study differed from the other experimental studies with respect to (a) the nature of the allergen, i.e., an LMW allergen instead of a HMW allergen; (b) the comparison between a minimally and distinctly irritating allergen concentration during challenge; (c) the concentration of the irritant, i.e., a high concentration to also induce effects in the lower respiratory tract; and (d) the comparison between short-term and long-term pre-exposure to the irritant. Overall, SO₂ exposure of TMA-sensitized BN rats reduced TMA-related allergy in most animals but facilitated TMA-induced irritation in a few animals. An explanation for the increased allergen-induced irritation is not easy to provide. The protection, i.e., the reduction in allergic symptoms, may be explained by the replacement of normal respiratory epithelium by less sensitive, squamous epithelium. In the present study, prolonged (10 days) SO₂ pre-exposure was more effective than short-time (1 day) pre-exposure, a finding that supported the hypothesis that the unexpected protection by SO₂ on TMA-induced

allergy was due to the induction of less responsive airway epithelium.

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