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Allergic inflammation in the upper respiratory tract of the rat upon repeated inhalation exposure to the contact allergen dinitrochlorobenzene (DNCB)

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ABSTRACT

Previously, the contact allergen dinitrochlorobenzene (DNCB) was identified as a sensitizer by inhalation in BALB/c mice; in addition, DNCB induced a lymphocytic infiltrate in the larynx of dermally sensitized Th1-prone Wistar rats upon a single inhalation challenge. In the present study, repeated inhalation exposures to DNCB were investigated using the same protocol as the single-challenge study: female Wistar rats were dermally sensitized with DNCB and subsequently challenged by inhalation exposure to 7 or 15 mg/m^3 DNCB twice a week for 4 weeks. Allergy-related apnoeic breathing was not observed. DNCB-specific IgG antibodies were found in the serum and – predominantly lymphocytic – inflammations were found in the nasal tissues and larynx. Similar effects were observed in animals repeatedly exposed by inhalation without previous dermal contact, indicating sensitization by inhalation. The inflammation may be the upper respiratory tract analogue of hypersensitivity pneumonitis/allergic alveolitis. Possible progression of the airway inflammation upon long-term exposure should be investigated to support or dismiss discrimination between contact and respiratory allergens in relation to respiratory allergy.

1. Introduction

Several low molecular weight (LMW) chemicals can cause contact allergy in the skin, but only a limited number of LMW allergens is known to cause respiratory allergy. Interestingly, all respiratory allergens tested in the murine local lymph node assay (LLNA) were positive upon skin application (Basketter and Scholes, 1992; Kimber et al., 2007), and thus are considered to sensitize the body via the dermal route. Based on human evidence, the skin appears more prone to Thelper1- and the respiratory tract more prone to Thelper2-mediated allergic disorders. However, in test animals the skin is considered a particularly effective route to sensitize the respiratory tract for Th2-mediated allergic reactions by LMW allergens (Arts and Kuper, 2003; Botham et al., 1989; Karol, 1986; Warbrick et al., 2002b), and there is evidence that this is true in humans as well (Redlich and Herrick, 2008). Various animal models for respiratory allergy have therefore been developed which exploit this capacity, and depend on dermal sensitization followed by an inhalation challenge. The major advantage of separation of the routes of sensitization (a systemic process) and challenge (local reaction), is that effects of the challenge only can be investigated without interference by previous sensitization via the same route.

Data concerning the risk of inhalation exposure to Thelper1-mediated contact allergens are limited. Recently, the contact allergens DNCB and oxazolone (de Sousa and Parrott, 1969; Kimber and Weisenberger, 1989) were found positive in a respiratory LLNA, based on a stimulation index (SI) of 3 or more in the respiratory tract-draining lymph nodes (Arts et al., 2008). They could therefore be considered capable of sensitization by inhalation. In addition, both contact allergens induced a mixed Th1/Th2 cytokine profile in the respiratory tract-draining lymph nodes (de Jong et al., 2009), comparable to the cytokine profiles induced by skin application (Dearman et al., 2003; Ulrich et al., 2001; van Och et al., 2002). Guinea pigs, challenged by inhalation exposure to 10 mg/m^3 DNCB, did not exhibit any changes in breathing frequency but demonstrated slightly elevated levels of DNCB-specific IgG1 (Botham et al., 1989). Furthermore, inhalation challenge with 7.5 or 27 mg/m^3 DNCB did not provoke allergy-associated breathing changes or allergic inflammation in the Th2-prone Brown Norway (BN) rat, despite upregulation of several allergy-associated genes (Kuper et al., 2008). However, it did induce a minimal lymphocytic infiltrate in the larynx of dermally sensitized Th1-prone Wistar rats (Arts et al., 1998).

Abbreviations: BAL, bronchoalveolar lavage; BALT, bronchus-associated lymphoid tissue; BN, Brown Norway; DNCB, 2,4-dinitrochlorobenzene; LLNA, local lymph node assay; LMW, low molecular weight.

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The above-mentioned challenge studies demonstrated that a single inhalation challenge with the contact allergen DNCB in sensitized Wistar rats induced minimal allergic inflammation in the respiratory tract, but no distinct functional breathing changes. However, repeated inhalation exposure to DNCB might have more serious consequences. In the present study, we therefore used the low-IgE-responding, Th1-prone Wistar rat to investigate whether repeated inhalation exposures to DNCB could induce breathing changes indicative of respiratory allergy and a more vigorous airway inflammation than observed following a single inhalation challenge exposure. To that end, the study protocol that successfully identified chemical and protein respiratory allergens in the past using a single inhalation challenge (Arts et al., 1998; Arts and Kuper, 2003; Pauluhn et al., 2002; Saloga et al., 1994; Zhang et al., 2004), was extended with repeated inhalation exposures. The allergic and irritating responses were studied by analyzing breathing parameters in exposed animals; IgE and hapten-specific IgG levels in serum; histopathology of the airways; and cellular and biochemical changes in bronchoalveolar lavage (BAL) fluid.

2. Material and methods

2.1. Animals and maintenance

Female, 8-week-old Wistar WU rats (Crl:[WI]WU BR, outbred) were purchased from a colony maintained under SPF conditions at Charles River Deutschland (Sulzfeld, Germany) and acclimatized for 13 days before the start of the study. They were kept under conventional laboratory conditions (3 animals per cage), and received a commercially available rodent diet (Rat & Mouse No. 3 Breeding Diet RM3; SDS Special Diets Services, Witham, England) and unfluoridated tap water ad libitum. Mean bodyweight (±standard deviation) was 189 (±7) g at the start of the study. All animal procedures were approved by the TNO Committee on Animal Welfare.

2.2. Materials

2,4-Dinitrochlorobenzene (DNCB; CAS No. 97-00-7; purity at least 98%) was obtained from Sigma (St. Louis, MO, USA); acetone from Biosolve (Valkenswaard, The Netherlands) and raffinated olive oil was supplied by Sigma (St. Louis, MO, USA).

2.3. Study design

The study design was based on a protocol which has successfully identified chemical and protein respiratory allergens in the past, using a single inhalation challenge. In short, 4 days before the first chemical application, rats were shaved on both flanks with an electrical razor and blood was sampled by orbital puncture for measurement of baseline levels of IgE and DNCB-specific IgG. Groups of rats (6 animals per group) received 150 μl 1% (w/v) DNCB in a vehicle consisting of a 4:1 (v/v) mixture of acetone and olive oil (AOO) on each shaved flank (approximately 12 cm^2) at day 0. Seven days later, they received 75 μl 0.5% (w/v) DNCB on the dorsum of each of both ears. Another set of animals was exposed by inhalation only, without previous dermal contact. Starting on day 21, rats were challenged by inhalation exposure to 7 or 15 mg/m^3 DNCB for 15 min, twice a week for 4 weeks (on days 21, 24, 27, 30, 34, 37, 41 and 43). Challenge concentrations were based on a previous experiment using a single inhalation exposure (Arts et al., 1998). Basal respiration parameters (breathing frequency, tidal volume and breathing pattern) were monitored of 2 animals per group on day 20 and subsequently during and after all successive challenges. Necropsy was performed on day 44, blood was sampled, bronchoalveolar lavage was performed, organs were weighed (liver, kidneys and left lung), and the ears and respiratory tract were collected for histopathological examination. An outline of the treatment schedule is depicted in Table 1.

Table 1
Treatment schedule.

Group designation	Sensitization Day 0: 150 μl per flank Day 7: 75 μl per ear	Challenge 15 min inhalation Days 21–43[a]
−/Low	Vehicle[b]	7 mg/m^3 DNCB
−/High	Vehicle[b]	15 mg/m^3 DNCB
+/Low	1% DNCB (day 0) 0.5% DNCB (day 7)	7 mg/m^3 DNCB
+/High	1% DNCB (day 0) 0.5% DNCB (day 7)	15 mg/m^3 DNCB

Respiration was monitored before the first, and during and after all successive challenges. At necropsy on day 44, animals were examined for gross pathological changes, blood was sampled (IgE and DNCB-specific IgG), BAL was performed, organs were weighed (liver, kidneys and left lung), and the nose, larynx, trachea, left lung and ears were collected for histopathological examination.

[a] On days 21, 24, 27, 30, 34, 37, 41 and 43.

[b] Acetone and olive oil in a 4:1 (v/v) mixture (AOO).

2.4. Atmosphere generation and analysis

A compressed air driven atomizer (Schlick type 970/S, Coburg, Germany) was used to generate liquid aerosols from freshly prepared solutions of DNCB. Airflow to the nebulizer was monitored by a mass stream meter (Bronkhorst Hi Tec, Ruurlo, The Netherlands). DNCB was dissolved in acetone to a concentration of approximately 18 g/l and delivered to the nebulizer by a motor-driven syringe pump (WPI type SP220i, World Precision Instruments, Sarasota, FL, USA). The acetone concentration was kept below 1 g/m^3, which is considered to be far below a level inducing sensory irritation (Alarie, 1973; de Ceaurriz et al., 1981; Schaper and Brost, 1991). Samples of the test atmosphere were collected prior to or after inhalation exposure and DNCB concentrations were determined gravimetrically by filter sampling at 5 l/min. Average actual concentrations (±standard deviation) of DNCB during inhalation exposure were 7.29 (±0.59) and 15.47 (±1.12) mg/m^3 for the low and high exposure groups, respectively. Particle size distributions of DNCB in the test atmospheres were determined using a 10-stage cascade impactor (Andersen, Atlanta, GA, USA) and the Mass Median Aerodynamic Diameter (MMAD) and geometric standard deviation (gsd) were calculated (Lee, 1972). MMAD and gsd were 2.7 μm and 4.4 in the 7 mg/m^3 DNCB atmosphere, and 2.4 μm and 4.6 in the 15 mg/m^3 DNCB atmosphere, respectively. Temperature and relative humidity (RH) were recorded during exposure; the average temperature was 21.7 ± 0.3 °C for the low exposure and 22.3 ± 0.3 °C for the high DNCB exposure groups. The average RH was 50.4 ± 2.8% and 50.3 ± 1.9% for low and high DNCB concentrations, respectively. Airflow through the exposure unit was 23 l/min.

2.5. Lung function measurements

Animals were exposed to the test atmosphere and lung function was measured as previously described (Arts et al., 1998). Groups of 12 rats were exposed simultaneously in a head/nose-only inhalation unit. The exposure unit permitted monitoring of the respiration of 4 of 12 animals (2 DNCB sensitized and 2 vehicle-treated controls), which were restrained in Battelle tubes and placed individually into one of four plethysmographs that were connected to the central exposure chamber. Thus, of each group of animals, the same two rats were used to analyze lung function during and after all inhalation exposures. Each plethysmograph was provided with a pressure transducer which sensed changes created by in- and expiration and transmitted amplified signals to a polygraph recorder, so respiratory frequency and pattern could be determined. Using this experimental setup, normal breathing pattern of the animals, exposed to fresh air, was assessed 1 day before the first inhalation exposure. Subsequently, respiration was monitored continuously during the 15 min inhalation challenge periods, 20 s/min for 15 min after each challenge, and 20 s/min for 6 min 1 day after each challenge. Mean values (breathing frequency, tidal volume and minute ventilation) were obtained from 6 exposure and 6 post-exposure values.

2.6. Clinical signs, body and organ weights, serum collection, and necropsy

All animals were observed at least once daily and weighed weekly, shortly before the start of the experiment and prior to necropsy. Serum samples were prepared from blood withdrawn via the orbital plexus prior to sensitization, and via the abdominal aorta at necropsy. Serum samples were stored at −20 °C until analysis of total serum IgE and DNCB-specific IgG levels by means of ELISA. At necropsy, animals were anaesthetized with sodium pentobarbital (ip) followed by exsanguination from the abdominal aorta, and examined grossly for abnormalities. Liver, kidneys and the unlavaged left lung (see below) were weighed. The nasal tissues, larynx, trachea, left lung and ears (site of dermal application) were collected for histopathological evaluation.

2.7. Bronchoalveolar lavage

After binding of the left lobe at necropsy, which was used for histopathological evaluation, the right lung lobes were lavaged (Hooftman et al., 1988) two times with a volume of 23 ml saline per kg bw. The total amount of retracted lavage fluid was weighed and retained on ice. The bronchoalveolar cells were isolated from the supernatant by centrifugation (250 g) during 5 min at 4 °C and resuspended in 0.5 ml saline to assess total 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 microscopy. For differential cell counts, cytospins 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 (Bradford, 1976), lactate