

Specific Inhalation Challenge in Persulfate Asthma

O. Hagemeyer, E. Marek, V. van Kampen, I. Sander, M. Raulf, R. Merget, and T. Brüning

Abstract

Specific inhalation challenge (SIC) may be considered the ‘gold standard’ for the diagnosis of occupational asthma due to persulfate salts. The aim of the study was to develop a safe SIC protocol. Between 2003 and 2014, eight patients with suspected occupational asthma due to persulfate salts were examined (7 females, all hair-dressers). SIC was done with a dosimeter and a nebulizer using ammonium persulfate dissolved in phosphate buffer. Until 2009, a four-step-protocol (doses: 0.0004, 0.0045, 0.045, 0.45 mg; cumulative: 0.5 mg) was used, afterwards a six-step-protocol (doses: 0.0004, 0.0018, 0.007, 0.028, 0.113, 0.45 mg; cumulative: 0.6 mg). With each SIC protocol, four subjects were tested. Skin prick tests with ammonium persulfate (20 mg/mL) were performed in all and patch tests in four subjects. In total, four subjects showed a positive SIC, two with each protocol. All subjects showed an isolated late reaction. The greatest decrease of volume in 1 s was 35 % about 3.5 h after the last inhalation (four-step-protocol). Skin prick test with ammonium persulfate was positive in one SIC positive (2 mm wheal) and in two SIC negative patients (3 and 4 mm wheal). All four subjects tested with patch tests showed a positive reaction; three of them were SIC_{pos}. We recommend to include patch-testing in the diagnosis of suspected occupational asthma due to persulfate salts. Isolated late asthmatic reactions may occur after SIC. The proposed six-step SIC protocol was safe in this limited number of subjects.

Keywords

Hairdressers • Occupational asthma • Occupational disease • Patch test • Skin prick test

O. Hagemeyer (✉), E. Marek, V. van Kampen, I. Sander, M. Raulf, R. Merget, and T. Brüning
Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-University Bochum (IPA), Bürkle-de-la-Camp-Platz 1, D-44789 Bochum, Germany
e-mail: hagemeyer@ipa-dguv.de

1 Introduction

Persulfate salts are low-molecular-weight allergens and may induce rhino-conjunctivitis, asthma, contact urticarial, and anaphylaxis (Hoekstra et al. 2012; Hougaard et al. 2012). Predominantly hairdressers are exposed to persulfate salts during hair bleaching (Moscato et al. 2005; Merget et al. 1994), but epidemiological data of allergic diseases due to persulfate are rare. From 2007 to 2012 at least 148 patch tests with ammonium persulfate were found positive (18.7 %) in 696 hairdressers with suspected contact dermatitis in Germany (Uter et al. 2014).

So far the pathogenesis and the value of diagnostic tests (e.g., skin prick test) in persulfate asthma are still unclear. Therefore specific inhalation challenges (SICs) are considered the 'gold standard' in the diagnosis of persulfate asthma. Standardized SICs with persulfate have been described previously. Munoz et al. (2003, 2004) used potassium persulfate powder in increasing concentrations (5, 10, 15, and 30 g mixed in 150 g lactose) and the tested subject had to tip powder from one tray to another. Moscato et al. (2005) nebulized ammonium persulfate dissolved in distilled water (mean concentration: 1.01 ± 0.11 mg/ μ L) and performed a one-step SIC lasting 30 min.

Since 2003, eight patients with suspected occupational asthma due to persulfate were examined in our outpatient clinic. All of the patients underwent a SIC with one of two newly developed very similar protocols.

2 Methods

The Ethics Committee of the Ruhr-University Bochum gave approval to perform the study. All patients transferred to our outpatient clinic with suspected occupational asthma due to persulfate between 2003 and 2014 were included in this retrospective analysis (Patient 3 had been presented by Harth et al. (2006), Polychronakis et al. (2013) presented patient 6). All patients answered a questionnaire for medical history

and underwent a physical examination, laboratory routine parameters including blood cell count, total immunoglobulin E (IgE), electrocardiogram, exhaled nitric oxide (eNO; Niox Mino, Aerocrine, Solna, Sweden), and X-ray of the thorax.

Lung function measurements (spirometry and body plethysmography; Carefusion, Würzburg, Germany) were performed according to the guidelines of the American Thoracic Society (ATS). The European Community for Steel and Coal (ECSC) reference values for spirometry were chosen (Quanjer et al. 1993). Methacholine testing was performed with a reservoir method as described by Polychronakis et al. (2013). Bronchial hyperresponsiveness was assumed if (i) forced expiratory volume in 1 s (FEV₁) was reduced ≥ 20 % or (ii) specific airway resistance (sRt) doubled and increased to ≥ 2.0 kPa/s with a cumulative dose of ≤ 300 μ g methacholine. Sputum induction with 0.9 % saline and processing of sputum samples were done as described by Raulf-Heimsoth et al. (2011). Skin prick tests were performed in all subjects with 20 environmental allergens and with ammonium persulfate (20 mg/mL, Sigma-Aldrich, Seelze, Germany). A wheel diameter of at least 2 mm was defined as positive. Patch testing was performed with 2.5 % ammonium persulfate (HAL; Düsseldorf, Germany) in four subjects. The patch remained on the back for 24 h, reactions were read at 24, 48, and 72 h.

SIC was carried out on 1 day with an APSpro dosimeter (Carefusion, Würzburg, Germany) combined with a 646 DeVilbiss nebulizer. Ammonium persulfate was freshly dissolved in phosphate buffer for all tests. Until 2009 (subjects 1–4), a four-step protocol was used. Each dose of ammonium persulfate was administered by inhalation of five slow deep breaths from functional residual capacity to near total lung capacity. During each breath the nebulizer acted for 0.6 s with an output of about 900 mg/min and an inspiratory airflow of about 1 L/s. Tenfold concentrations of 0.01, 0.10, 1.00, and 10.00 mg ammonium persulfate per mL were used resulting in doses that increased from 0.40 μ g to 0.45 mg, with a cumulative dose of 0.50 mg (SIC protocol I).

Table 1 Personal data of 8 patients with suspected occupational asthma due to ammonium persulfate

	Four-step-protocol				Six-step-protocol			
	Pat 1	Pat 2	Pat 3	Pat 4	Pat 5	Pat 6	Pat 7	Pat 8
Gender	f	f	f	f	f	m	f	f
Age (years)	44	30	27	24	39	32	20	46
Atopy	no	yes	yes	yes	no	yes	yes	yes
Smoking	yes	yes	yes	yes	yes	yes	never	never
Exposure time (months)	288	60	108	84	264	15	36	284
Latency period ^a (months)	240	2	96	24	216	0	24	12
Exposure time with symptoms (months)	48	58	1	60	48	15	6	48
Time between end of exposure and examination (months)	12	14	7	7	7	6	12	8
Asthma medication	yes	yes	yes	yes	no	yes	yes	yes

PAT patient

^aTime between starting exposure and development of symptoms

Since 2010, a six-step-one-day SIC with quadrupling doses (SIC protocol II) was used (subjects 5–8). The inhalation procedure was the same as in protocol I, but ammonium persulfate concentrations were applied in quadrupling concentrations (0.010, 0.039, 0.156, 0.625, 2.500, and 10.000 mg/mL), doses increased from 0.40 µg to 0.45 mg, with a cumulative dose of 0.60 mg.

According to both SIC protocols, after inhalation of saline patients inhaled the ammonium persulfate solution with time-lags of 10 min. Lung function was measured before SIC, after each inhalation step, 30, 60, and 120 min and about 24 h after SIC. The measurements of bronchial hyperresponsiveness, eNO, and sputum analysis were performed before and about 24 h after SIC. Anti-obstructive medication was withheld at least 24 h before SIC.

As positivity criteria of SIC we used the same lung function changes from baseline as for methacholine testing after any ammonium persulfate dose. A late reaction was defined as a positive reaction beginning later than 2 h after the end of exposure.

Since 2010, a six-step-one-day SIC with quadrupling doses (SIC protocol II) was used (subjects 5–8). The inhalation procedure was the same as in protocol I, but ammonium persulfate concentrations were applied in quadrupling concentrations (0.010, 0.039, 0.156, 0.625, 2.500, and 10.000 mg/mL), doses increased from 0.40 µg to 0.45 mg, with a cumulative dose of 0.60 mg.

Three patients showed a positive SIC, two of them with the four-step protocol. Patient 5, who underwent a six-step SIC, showed a FEV₁ decrease of 18 % 2 h after the last inhalation step and was defined positive, too (overall 4 positive SICs). In all cases, the reaction was a late one (Fig. 1a). Patient 1 was diagnosed with occupational asthma due to persulfate 19 months earlier in another hospital, where she underwent a SIC with persulfate and showed a positive reaction. This positive reaction could not be reproduced with our test (overall 4 negative SICs).

The latency period (time between starting exposure and first symptoms) in SIC positive women (including patient 1) was 2 to 240 months and in SIC negative 12 to 24 months. Total exposure time was 60 to 264 months in SIC positive women and 16 to 84 months in SIC negatives. The only man (SIC positive) was exposed for 15 months and his symptoms appeared shortly after he had started to work. At the time of examination all but one patient took antiobstructive medication (Table 1). Work-related symptoms of the nose and skin were

3 Results

Eight patients (7 women, 1 man) with suspected occupational asthma due to persulfate were examined between 2003 and 2014. While all

Fig. 1 (a) Time-response curve of FEV₁ during specific inhalation challenge (SIC). The near-positive immediate type reaction was considered negative due to insufficient breathing technique and lack of symptoms and change of specific airway resistance. (b) Serial measurements of eNO; (c) sputum eosinophils; and (d) methacholine responsiveness all performed before and 24 h after SIC

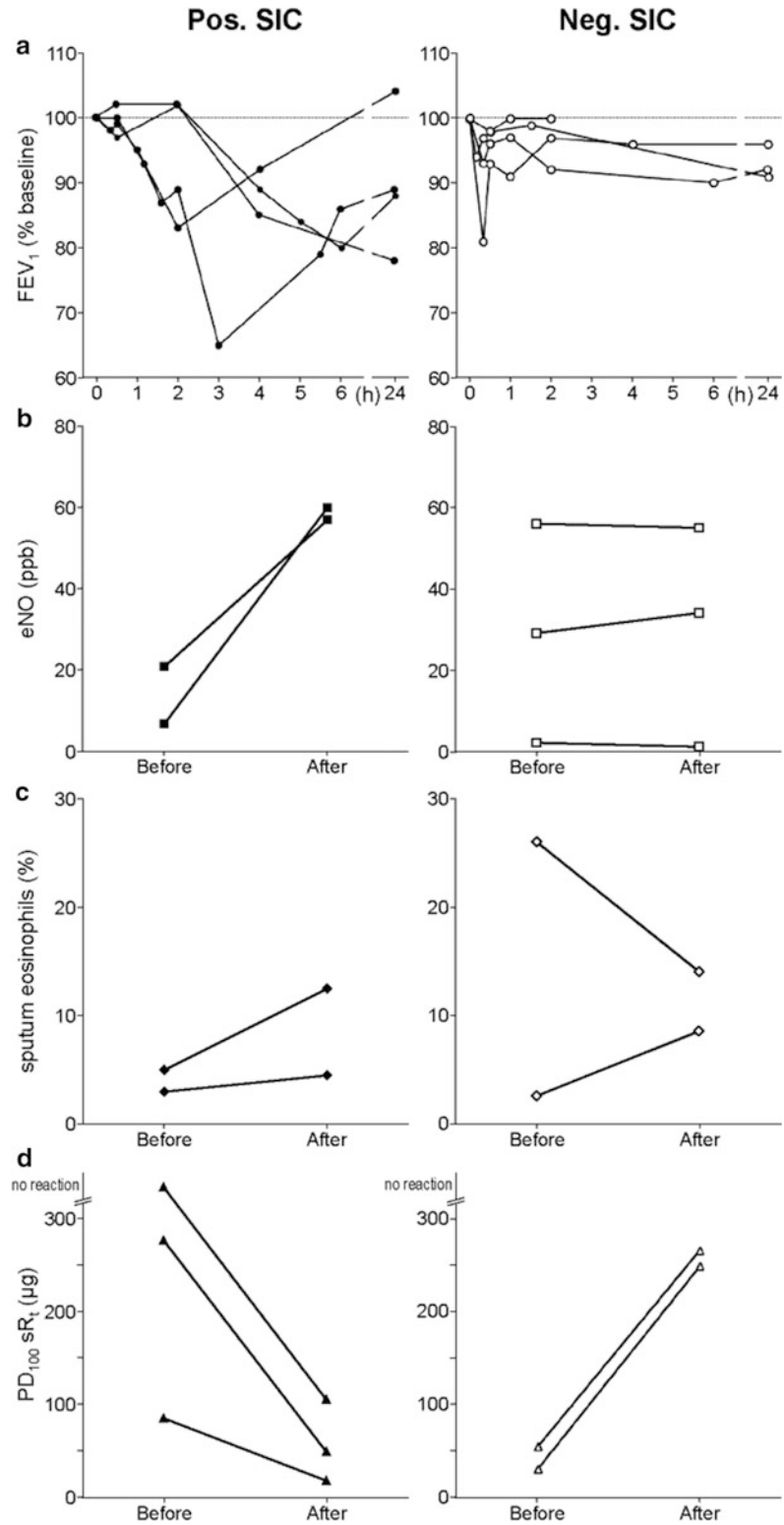


Table 2 Work-related symptoms and diagnostic findings

	Four-step-protocol				Six-step-protocol			
	Pat 1 ^a	Pat 2	Pat 3	Pat 4	Pat 5	Pat 6	Pat 7	Pat 8
Symptoms								
Skin	yes	yes	no	no	yes	yes	no	yes
Eyes	no	no	no	no	no	no	no	no
Nose	yes	yes	yes	yes	yes	yes	no	yes
Cough	yes	no	no	no	no	no	yes	yes
Phlegm	yes	no	no	no	no	no	no	no
Dyspnea	no	yes	yes	yes	yes	yes	yes	yes
Diagnostic findings								
FEV ₁ (%pred.)	84	91	100	100	108	89	106	90
Methacholine challenge								
PD ₁₀₀ sR _t	55	41	278	neg.	neg.	62	neg.	52
PD ₂₀ FEV ₁	162	48	215	104	neg.	153	neg.	56
Sputum eosinophils (%)	0	5	10	3	2	6	1	7
Total IgE (kU/L)	172	575	133	273	228	702	127	40
SPT with APS (wheal and flare, mm)	neg.	2 (10)	neg.	neg.	neg.	neg.	4 (10)	3 (3)
Patch test with APS	n.d.	n.d.	pos.	n.d.	pos.	pos.	n.d.	pos.
SIC with APS	neg.	late	late	neg.	late	late	neg.	neg.

PAT patient, PD₂₀FEV₁ a 20 % fall in forced expiratory volume in 1 s (FEV₁), PD₁₀₀sR_t doubling of specific airway resistance, SPT skin prick test, APS ammonium persulfate, SIC specific inhalative challenge, n.d. not done

^aDiagnosis of occupational asthma due to APS was based on SIC outcome in another clinic some months before

frequent (Table 2). Lung function testing was normal in all patients, but only two SIC negative patients did not show bronchial hyperresponsiveness.

Total IgE concentrations were elevated in all but one SIC negative patients (normal: <100 kU/L) (Table 2). Blood eosinophils were increased to ≥ 5 % in all but two SIC negative patients (data not shown). Skin prick test with ammonium persulfate was positive in one SIC positive and in two SIC negative atopic women. Patch tests were performed in four patients, all with a positive reaction. Three of them were SIC positive. SIC was performed in each patient (Fig. 1). During SIC, two patients (no. 2 and no. 3) reported runny nose. All positive tests were late bronchial reactions. The highest decrease of FEV₁ of 35 % was seen about 3.5 h after the end of exposure. Three patients were given antiobstructive medication (patients 3, 6, both SIC positive; patient 8, SIC negative).

Serial measurements of eNO were performed in 5 patients (Fig. 1b). In two SIC positive, but in

no SIC negative patient, there was a significant increase in eNO after SIC. Eosinophils in induced sputum were analyzed in four subjects. In three cases there was an increase in eosinophils (including one SIC negative patient) (Fig. 1c). Bronchial hyperresponsiveness increased in three of the four patients (one was not examined). In SIC negative patients there was an increase of bronchial reactivity (Fig. 1d).

4 Discussion

SICs are generally considered as the ‘gold standard’ in the diagnosis of occupational asthma. This is especially true for persulfate allergy because there is no *in vitro* test available for the demonstration of sensitization (Hougard et al. 2012; Moscato et al. 2005). Symptoms at work cannot predict the existence of occupational asthma either (Moscato et al. 2005; Blainey et al. 1986).

Munoz et al. (2003, 2004) and Moscato et al. (2005) have previously performed semi-quantitative SICs with persulfate, but they exposed their patients to unknown, probably high concentrations. To our knowledge, so far no quantitative test protocols allowing the calculation of the dose required for a positive reaction have been published. This would be important for both immediate and isolated late reactions in order to avoid too low or unnecessarily high maximal doses, with the risk of false positive or severe asthmatic reactions. We herein present a multiple-step, one day SIC protocol to diagnose occupational asthma due to ammonium persulfate with increasing exposure concentrations from 0.01 to 10 mg/mL and cumulative doses of 0.5 or 0.6 mg, respectively.

Skin prick test with persulfate, mostly performed with ammonium persulfate, may show immediate type reactions (Munoz et al. 2003, 2004, 2008; Wrbitzky et al. 1995; Parra et al. 1992), but in most cases late reactions are induced. Moscato et al. (2005) noted 14 isolated late reactions in 21 SIC positive patients and Munoz et al. (2004) 6 late reactions in 8 positive SICs. Also Blainey et al. (1986) saw only late reactions ($n = 15$). This is in accordance with our present results.

In the present study, one patient showed a decrease of FEV_1 of about 35 % (patient no. 3) while in the other cases the decrease was smaller. In the study of Munoz et al. (2003), patients handled a mixture of 150 g lactose and 5 g potassium persulfate powder and the measured air levels of total dust ranged from 1 to 6 mg/m³. In 5 of 8 positive SICs, a fall of FEV_1 of about 30–40 % was induced. Moscato et al. (2005) nebulized persulfate solution in a concentration of about 1.01 ± 0.11 mg/ μ L. In their study the maximum decrease of FEV_1 was also about 40 % (three patients). We assume that the presented six-step SIC protocol is less aggressive and ensures a higher security to the patients to prevent excessive FEV_1 decreases.

The early four-step protocol with ten-fold dilutions was replaced by a six-step protocol with quadrupling doses due to safety reasons. Although the lowest and highest single doses

were identical in both protocols, the cumulative dose was marginally higher in the six-step protocol. Overall, the observed degrees of airways obstruction were considered acceptable. Due to the limited number of tests, further studies will be required in order to estimate whether the proposed protocol indeed will be sufficiently safe.

In four patients of the present study, SIC was negative. Patient no. 1 had shown a positive SIC reaction some months ago in a previous external examination and was therefore diagnosed as occupational asthma due to persulfate. We do not know any details of this test which was performed by a 15-min handling of bleaching powder. It is possible that the patient became negative in our examination due to the time lapse from the external SIC. Improvement of occupational asthma due to persulfate after avoiding exposure was described in literature (Munoz et al. 2008). Patient no. 8 was sensitized to ammonium persulfate and showed bronchial hyperresponsiveness, but had a negative SIC.

Concerning the sensitivity of our SIC protocols, we cannot exclude false negative reactions, but in view of the high maximal fall in FEV_1 , the increase of the maximal dose should be performed cautiously, possibly a 2-day protocol should be considered. We have not performed SICs with ammonium persulfate in controls, but the negative SICs in patients with bronchial hyperresponsiveness and the lack of an immediate reaction indicate high specificity. An association was seen between a positive patch test and a positive SIC. On the other hand, there were no associations between the results of SIC and smoking, atopy, or skin prick test with ammonium persulfate. This is in accordance with other studies (Moscato et al. 2005). As a result of our findings we recommend to include patch testing in the diagnostic procedure, which has rarely been reported in the literature.

We also tested parameters indicating airways inflammation before and after SIC. The interpretation of the results of these tests (serial testing of eNO, sputum eosinophils, and bronchial hyperresponsiveness) is limited by a considerable number of missing values. However, there was an overall concordance between

eosinophilic airway inflammation and SIC results. We consider these additional effect parameters especially useful for the detection of isolated late reactions which are sometimes difficult to document. These tests also indicate that isolated late reactions may be mediated by eosinophilic inflammatory processes.

Conflicts of Interests The authors declare no conflict of interest in relation to this article.

References

- Blainey AD, Ollier S, Cundrell D, Smith RE, Davies RJ (1986) Occupational asthma in a hairdressing salon. *Thorax* 41:42–50
- Harth V, Raulf-Heimsoth M, Brüning T, Merget R (2006) Isolated late asthmatic reaction after exposure to ammonium persulfate in a hairdresser. *Contact Dermatitis* 54:62–63
- Hoekstra M, van der Heide S, Coenraads PJ, Schuttelaar ML (2012) Anaphylaxis and severe systemic reactions caused by skin contact with persulfates in hair-bleaching products. *Contact Dermatitis* 66:317–322
- Hougaard MG, Menné T, Sosted H (2012) Occupational eczema and asthma in a hairdresser caused by bleaching products. *Dermatitis* 23:284–297
- Merget R, Buenemann A, Kulzer R, Rueckmann A, Breitstadt R, Kniffka A, Kratisch H, Vormberg R, Schultze-Werninghaus G (1994) A cross sectional study of chemical industry workers with occupational exposure to persulfate. *Occup Environ Med* 53:422–426
- Moscato G, Pignatti P, Yacoub MR, Romano C, Spezia S, Perfetti L (2005) Occupational asthma and occupational rhinitis in hairdressers. *Chest* 128:3590–3598
- Munoz X, Cruz MJ, Orriols R, Bravo C, Espuga M, Morell F (2003) Occupational asthma due to persulfate salts. *Chest* 123:2124–2129
- Munoz X, Cruz MJ, Orriols R, Torres F, Espuga M, Morell F (2004) Validation of specific inhalation challenge for the diagnosis of occupational asthma due to persulfate salts. *Occup Environ Med* 61:861–866
- Munoz X, Gómez-Ollés S, Cruz MJ, Untoria MD, Orriols R, Morell F (2008) Course of bronchial hyperresponsiveness in patients with occupational asthma caused by exposure to persulfate salts. *Arch Bronchopneumol* 44:140–145
- Parra FM, Igea JM, Quirce S, Ferrando MC, Martin JA, Losada E (1992) Occupational asthma in a hairdresser caused by persulphate salts. *Allergy* 47:656–660
- Polychronakis I, Thanasis E, Raulf-Heimsoth M, Merget R (2013) Occupational non-immediate type allergic asthma due to ammonium persulfate. *Adv Exp Med Biol* 755:79–83
- Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC (1993) Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J* 16:5–40
- Raulf-Heimsoth M, Pesch B, Kendzia B, Spickenheuer A, Bramer R, Marczyński B, Merget R, Brüning T (2011) Irritative effects of vapours and aerosols of bitumen of the airways assessed by non-invasive methods. *Arch Toxicol* 85:S41–S52
- Uter W, Gefeller O, John SW, Schnuch A, Geier J (2014) Contact allergy to ingredients of hair cosmetics – a comparison of female hairdressers and clients based on IVDK 2007–2012 data. *Contact Dermatitis* 71:13–20
- Wrbitzky R, Drexler H, Letzel S (1995) Early reaction type allergies and diseases of the respiratory passages in employees from persulphate production. *Int Arch Occup Environ Health* 67:413–441