Acute Effects of a Fungal Volatile Compound

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OBJECTIVE: 3-Methylfuran (3-MF) is a common fungal volatile product with active biologic properties, and previous studies have indicated a contribution to airway disease. The aim of the present study was to assess the acute health effects of this compound in humans.

DESIGN: Acute effects were assessed via chamber exposure to (1 mg/m3) 3-MF.

PARTICIPANTS AND MEASUREMENTS: Twenty-nine volunteers provided symptom reports, ocular electromyograms, measurement of eye tear film break-up time, vital staining of the eye, nasal lavage, acoustic rhinometry, transfer tests, and dynamic spirometry.

RESULTS: No subjective ratings were significantly increased during exposure. Blinking frequency and the lavage biomarkers myeloperoxidase and lysozyme were significantly increased, and forced vital capacity was significantly decreased during exposure to 3-MF compared with air control.

CONCLUSIONS AND RELEVANCE TO CLINICAL PRACTICE: Acute effects in the eyes, nose, and airways were detected and might be the result of the biologically active properties of 3-MF. Thus, 3-MF may contribute to building-related illness.

KEY WORDS: 3-methylfuran, airway physiology, biomarker, building-related illness, fungi, hypersensitivity pneumonia, lung, microbial volatile organic compound (MVOC), mold. Environ Health Perspect 113:1775–1778 (2005). doi:10.1289/ehp.8193 available via http://dx.doi.org/ [Online 9 August 2005]
time in seconds was recorded from the last blink until a rupture in the precorneal film was observed. We also estimated tear film stability by recording the self-reported tear film break-up time. The subjects were asked to keep their eyes open, and the time was recorded until they felt an urge to blink, assuming that this feeling was the appearance of a dry spot on the cornea (Wieslander et al. 1999). Measurements of break-up time were performed on three occasions in each eye: before entering the chamber, at the end of exposure, and 4 hr after exposure.

**Vital staining of the eye.** We assessed epithelial damage to the cornea and conjunctiva using a semiquantitative method. We instilled 4 µL of a dye, lissamine green (1% in physiologic saline solution), into the lower conjunctival sac. After 1 min, the cornea and conjunctiva were examined by a binocular microscope with a slit lamp (Topcon SL1E), and each eye was given a score of 0–9 (Norn 1991). Vital staining was performed once, 4 hr after exposure.

**Nasal lavage.** We measured inflammatory markers in nasal lavage samples before, directly after, and at 2 hr postexposure. Lavage of the nasal mucosa was collected with a 20-µL plastic syringe attached to a nose olive (Wålinder 1999). The analyses included myeloperoxidase (MPO), eosinophil cationic protein (ECP), lysozyme, and albumin and were carried out at the Department of Clinical Chemistry, University Hospital, Uppsala, Sweden. The chemical analysis of lavage biomarkers has been described in detail elsewhere (Wålinder 1999).

**Transfer test.** We determined the diffusion capacity of carbon monoxide (DLCO) using a single-breath technique (transfer test; PK Morgan Ltd., Chatham, Kent, UK) (Cotes et al. 1997; Forster et al. 1954). DLCO was measured for each subject before entering the exposure chamber and 20 min after leaving the exposure chamber.

**Dynamic spirometry.** Dynamic spirometric measurements were performed for each subject before entering the exposure chamber, immediately after leaving it, and 2 hr after leaving the chamber. Spirometric tests included vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV₁), peak expiratory flow (PEF), and forced expiratory flow (FEF) in the middle half of FVC (FEF₂₅, 50, 75, the expiratory flows after one-fourth, one-half, and three-fourths, respectively, of the vital capacity has been expired [after a full inspiration]). The tests were performed by spirometry (Vitalograph 2120 and Spirotrac 3 software for PC, version 2.0; Vitalograph, Buckingham, UK) according to the guidelines prescribed by the American Thoracic Society (1995).

**Acoustic rhinometry.** We assessed nasal patency using acoustic rhinometry. The nasal volume (from the nostril and 7 cm into the nasal cavity) and the minimal cross-section area were determined as an average of three measurements in each nostril. We performed the rhinometric measurements for each subject at three occasions during the exposure day: before entering the chamber, immediately after leaving it, and 2 hr after leaving it. Data on the nasal volumes and areas were presented as the sum of the right and the left side. The rhinometer, using a single-click signal of audible frequencies with the Nasal Area-Distance Acquisition Program, version 1.0 (University of Aarhus, Aarhus, Denmark), has previously been described by Hilberg et al. (1989).

**Statistical methods.** We compared the differences before and after exposure to 3-MF and air control using t-tests for paired samples for rhinometric and spirometric changes and Wilcoxon matched pairs tests for the non-normally distributed lavage data. We used repeated-measures analysis of variance (ANOVA) for subjective ratings and the
blinking frequency series (Statistica for Windows, version 7.0; StatSoft Inc., Tulsa, OK, USA). Two-tailed tests and a 5% level of significance were applied when applicable.

Results

Suspected adverse reaction. We removed one subject from the exposure series because of a two-phased pulmonary reaction to 3-MF. During the last 30 min of exposure, the subject suffered from moderate airway distress combined with acute airway obstruction. The PEF fell from 320 L/min before exposure to 170 L/min directly after. The acute dyspnea cleared up quickly, and by 3 hr after exposure, the PEF was 350 L/min. Three days after exposure, the subject had an onset of severe chest tightness, together with chills, fatigue, cough, and fever around 39°C. One week after exposure, the subject suffered from leukopenia, and obstructive symptoms remained for about a month. The subject’s chest X-ray was normal, and no elevated titers for influenza virus A and B were found. Titers for total IgE and a mold-antigen panel were high. We reported the suspected adverse effect to the Regional Ethical Committee.

Symptom ratings. The symptom ratings were not different during exposure to 3-MF compared with clean air (Figure 1). An immediate weak odor detection of 3-MF could be seen among some of the subjects, but not all (Figure E). This suggests that the exposure level was near the odor threshold and that adaptation occurred.

Eye measurements. The blinking frequency during 3-MF exposure was significantly higher than during clean air exposure (Figure 2, Table 1). The vital staining scores of epithelial eye damage detected with lissamine green were slightly higher after 3-MF exposure, but this effect was not statistically significant. The tear-film break-up time was significantly higher at the end of the 2-hr exposure period compared with the air control (Table 1). The observed changes were similar in subjects with or without atopy.

Nasal measurements. We observed a washout effect with decreased biomarker concentrations after repeated lavages following exposure to air. In contrast, compared with air controls, we observed an increase that was significant for MPO directly after and for lysozyme 2 hr after exposure (Table 2). Nasal cavity dimensions, measured by acoustic rhinometry, were not different from air control (Table 3). Stratification by atopy did not show different reactivity for biomarkers or rhinometry, although baseline levels of ECP and albumin were doubled for subjects with atopy.

Airway measurements. On average FVC decreased 0.1 L directly after exposure to 3-MF, which was a significant decrease compared with air control. The other lung function parameters (transfer test, VC, FEV1, PEF) were not affected by exposure to 3-MF compared with clean air (Table 4). Stratification by atopy showed that the observed effect on FVC mainly appeared among nonatopics.

Discussion

Although the exposure level of 3-MF was near the smell threshold and did not cause subjective symptoms of mucosal irritation or airway distress, the objective measurements did show effects on the eyes and airways. Considering an increase of the blinking frequency as an indicator of eye irritation together with the...
nasal biomarker response, it is possible that 3-MF might have mucosal effects in both the eyes and the airways. We also found an increased tear film break-up time after exposure to 3-MF. The tear film stability is dependent on the quality and amount of the fatty layer on its surface that is produced from the meibomian glands. The secretion from these glands is stimulated by the blinking movements, and a congruent increase of both blinking frequency and break-up time can be expected.

MPO is a marker of the neutrophil activity in the nasal mucosa, and lysozyme is a marker of both neutrophil activity and secretory neurogenic stimuli. Because nasal lavage was performed three times, a washout effect with decreased concentrations could be expected. We observed this decrease for all lavage biomarkers after exposure to air in contrast to an increase after exposure to 3-MF. Also, the decreased FVC after exposure to 3-MF indicates an airway effect. This pulmonary function variable is slightly more sensitive to airway irritation and hyperreactivity than is the VC measurement with slow expiration.

3-MF is metabolically activated via microsomal oxidation, cleaving the furan ring to a reactive unsaturated dialdehyde, methylbutenenedial, that binds covalently to tissue macromolecules (Ravindranath et al. 1984). Animal inhalatory studies have revealed organ damage at high exposures. Haschek et al. (1984) reported that rats inhaling 1,000 mg/m³ 3-MF for 1 hr had damaged airway epithelium with pneumonitis and necrotizing suppurative rhinitis. They also observed necrosis, fibrosis, and epithelial metaplasia in the airways at autopsy 14 days later. Previous epidemiologic results also show airway reactions related to 3-MF in indoor air (Smedje et al. 1996).

This suspected adverse reaction was previously reported (Wålinder et al. 1998) in a subject with atopy who previously had been working in a mushroom farm and with microfungi. This subject suffered an acute obstructive reaction and a delayed pulmonary reaction with flulike symptoms. A nonspecific airway reaction could explain the immediate effects compared with persons who, because of long-term daily exposures, have acquired a form of sensitivity to “sick buildings.” Because persons with atopy are considered more sensitive to dampness, mold, or other disturbances of the indoor environment, subjects with IgE-mediated allergy to common allergens were recruited for the present study. However, results do not support the statement that persons with atopy report more symptoms or have a higher reactivity to this fungal metabolite. Actually, the only difference observed in reactivity was that nonatopics had a decrease in FVC after exposure to 3-MF, whereas no such effect was seen among the subjects with atopy.

In conclusion, we have recorded acute effects from the eyes, nose, and airways indicating mucosal reactive properties of 3-MF, which is commonly found in buildings affected by microbial growth. The mucosal effects could be induced by a possible chemical injury from the bioactivation of 3-MF. More unusual but severe effects, such as hypersensitivity reactions after exposure to fungi and molds, could also be explained by a protein-hapten reaction. Therefore, the results of the present study may have relevance for the judgment of health problems due to microbial emissions.