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ScienceDirect
Food and Chemical Toxicology 46 (2008) 527–536
www.elsevier.com/locate/foodchemtox

Subchronic inhalation of mixtures of cigarette smoke constituents in Xpa−/−p53+/− knock-out mice: A comparison of intermittent with semi-continuous exposure to acetaldehyde, formaldehyde, and acrolein

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Received 27 February 2007; accepted 23 August 2007

Abstract

We investigated whether inhaling peak concentrations of aldehydes several times daily is more damaging than semi-continuously inhaling low-dose aldehydes. We exposed Xpa−/−p53+/− knock-out mice either intermittently or semi-continuously to mixed acetaldehyde, formaldehyde, and acrolein. The intermittent regimen entailed exposure to the aldehydes 7 min every 45 min, 12 times/day, 5 days/week, corresponding to concentrations inhaled by smokers. Semi-continuously exposed animals received half the dose of aldehydes in 8 h/day, 5 days/week. Some mice in each group were sacrificed after 13 weeks of exposure; the rest breathed clean air until the end of 1 year. Mice injected intratracheally with benzo[a]pyrene formed a positive control group. The nasal cavity, lungs, and any macroscopically abnormal organs of all mice were analysed histopathologically. After 13 weeks of exposure, the subacute, overall, histopathological changes induced by the inhalation differed noticeably between the intermittently and semi-continuously treated Xpa−/−p53+/− knock-out mice. After 13 weeks of mixed aldehyde exposure, atrophy of the olfactory epithelium generally appeared, but disappeared after 1 year (adaptation and/or recovery). Respiratory epithelial metaplasia of the olfactory epithelium occurred at a higher incidence at 1 year. Except for a significantly greater number of tumours observed in knock-out mice compared to wild mice (semi-continuous aldehyde exposure and controls), no differences between the semi-continuous and intermittent exposure groups were observed.

Keywords: Acetaldehyde; Formaldehyde; Acrolein; Mice; Tumour; Xpa

1. Introduction

Aldehydes are ubiquitous indoor chemical pollutants known to be respiratory irritants with allergenic potential, for both animals and humans, as has recently been shown for formaldehyde, which causes sensitization and asthma aggravation even at low domestic exposure levels (Casset et al., 2006).

Cigarette smoke is another important source of aldehydes (Phillips and Waller, 1991). Aldehydes in cigarette

Abbreviations: ANOVA, analysis of variance; B[a]P, benzo[a]pyrene; SC, semi-continuous exposure to a mixture of acetaldehyde, formaldehyde and acrolein; IE, intermittent exposure to a mixture of acetaldehyde, formaldehyde and acrolein; KO, knock-out; MV, mean tidal volume; NOEL, no-effect level; PMN, polymorphonuclear neutrophils; Xpa, xeroderma pigmentosum complementation.

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doi:10.1016/j.fct.2007.08.043

smoke are combustion products from tobacco and arise especially from the added sugars. All aldehydes cause pathological damage to the respiratory tract and reach high peak concentrations in the respiratory tract during smoking. Exposure of rats to these aldehydes leads to a significant increase in damage to the respiratory tract and to a decrease in breathing frequency. The combined effect is, at most, the sum of the individual effects (Cassee et al., 1996a,b). However, it is uncertain whether the extent of the combined effects of cigarette smoking takes place during peak concentrations. Analysis of mainstream cigarette smoke shows that, among the aldehydes, acetaldehyde is the main component (about 700–1000 μg/cigarette), followed by acrolein and formaldehyde (60100 μg/cigarette), crotonaldehyde, diacetyl, and methyl glyoxal (IARC, 1986). Although charcoal filters adsorb 50–80% of the acrolein, side stream smoke contains about 12 times more acrolein than filtered smoke as a result of pyrolysis at a lower temperature (Thweatt et al., 2006).

Of the 4000–6000 components of cigarette smoke, aldehydes such as acrolein, formaldehyde, and acetaldehyde are among the most abundant congeners included in the list of 149 toxic components (Fowles and Dybing, 2003), and, particularly in the core 44 'Hoffmann analytes', they are considered relevant to smoking-related diseases (Baker, 2006). It has been recognized for a long time that aldehydes slow the ciliary beats in the respiratory tract (Kensler and Battista, 1963; Wynder et al., 1965). Furthermore, aldehydes cause 62.4% of the cancer risk attributable to cigarette smoke (Fowles and Dybing, 2003). Understandably, in view of its numerous toxic components, studies on inhalation of whole cigarette smoke cannot possibly evaluate the effects of aldehydes alone or even in combination.

Moreover, whereas most studies have focused on either its acute or chronic effects, cigarette smoking is intermittent, and exposure depends not only on the levels of the toxicants, but also on the frequency and duration; i.e. the pattern of cigarette smoking of each individual. Short bouts of aldehyde inhalation at high concentrations, mimicking real-life smoking conditions, are presently hypothesized to induce greater cytotoxic effects and pathological changes in the respiratory tract than semi-continuous inhalation exposure at lower concentrations.

The objectives of the present study were to simulate the conditions of cigarette smoking as closely as possible to study the health effects of inhaling a mixture of the three aldehydes acetaldehyde, formaldehyde, and acrolein on the respiratory tract of rodents (mice in particular) in both pattern of administration and concentration ratios comparable to those inhaled by humans. Mice appear to be a good animal model, as shown, for example, by studies of the respiratory irritative effects of formaldehyde (decreased respiratory rate linked to a trigeminal reflex), with a no-effect level (NOEL) of 0.3 ppm in mice, which is close to the human NOEL of 0.08 ppm (Nielsen et al., 1999). We compare the histopathologic effects induced by 13 weeks of intermittent exposure to high concentrations of the aldehydes, as observed during smoking, to the effects induced by 13 weeks of semi-continuous exposure to low aldehyde concentrations (equal total amounts per day) on two species of mice, the Xpa−/−p53+/− knock-out (KO) mice and C57BL/6 wild type mice, known to be sensitive to chemical toxicants. We studied the histopathology at 13 weeks and at 1 year (two cohorts of mice) to determine both the subacute and long-term effects of intermittent exposure to aldehydes, and compared the results to those from the negative and positive control groups exposed to either clean air or intratracheal benzo[a]pyrene (B[a]P), a known carcinogen in mouse and man (Hecht, 2005; Rojas et al., 2004).

2. Materials and methods

2.1. Chemicals

Formaldehyde, acetaldehyde, and acrolein were obtained form Janssen Chimica (Beerse, Belgium). The B[a]P was obtained from Sigma (St Louis, MO).

2.2. Animals

Following approval by the Ethical Committee of the National Institute for Public Health and the Environment, Bilthoven, The Netherlands, specific pathogen-free p53+/−/Xpa−/− mice were selected for the study, as these are known to be sensitive to chemical compounds. Xpa−/− KO refers to xeroderma pigmentosum complementation (group D) knock-out, and these mice are repair deficient in nucleotide excision with increased local and systemic immunosuppression (Garssen et al., 2000). All of the mice were in a C57BL/6 background. De Vries et al. (1995) describe the generation of Xpa mice. Tyler Jacks (MIT, Cambridge, MA) kindly provided the P53 mice (Jacks et al., 1994). Xpa mice were crossed with p53+/− mice to obtain Xpa/p53+/− mice. In total, 120 Xpa KO mice and 72 wild-type mice were used for these studies. The animals were obtained from GPL (Joint Centre for Laboratory Animals Studies Facility, RIVM/NVI, Bilthoven, The Netherlands). They were weighed upon arrival and every week thereafter, and they were randomly allocated to the various exposure groups. There were equal numbers of males and females in each group. During the acclimatization period of 1 week and the non-exposure periods, the animals were housed under conventional conditions, in groups of maximally five animals in stainless steel cages with wire-screen bottoms and fronts. Room temperature was maintained at 22 ± 2 °C and relative humidity, at 40–70% with a 12-h light/dark cycle. The animals were fed SSP-TOX (Hope Farms, The Netherlands) cereal-based pellets and had tap water *ad libitum*.

2.3. Aldehyde dose extrapolation from human to mouse

Given the mean aldehyde amounts inhaled per cigarette (acetaldehyde 1000 μg, formaldehyde 100 μg, and acrolein 100 μg), and assuming that these values were determined with the Federal Trade Commission method (FTC, 1997) under standardized conditions, with an average of 10 puffs and total smoke volume of 350 ml (10 × 35 ml) per cigarette, the smoke of one cigarette can be estimated to contain acetaldehyde at 1587 ppm (2.86 g/m^3), formaldehyde at 238 ppm (0.29 g/m^3), and acrolein at 124 ppm (0.29 g/m^3). These concentrations were, however, believed to be too high for mice, and lower levels were chosen. To obtain the same or similar aldehyde concentrations in mice lungs as in human lungs when people smoke, several calculations have to be made. First, given that the surface of human lungs corresponds to 50 m^2, a total of 1000 μg of acetaldehydes per cigarette will correspond to 20 μg/m^2 in human lungs. A mouse has a lung surface area of 0.12 m^2, so that the dose required to obtain a similar exposure (21 μg/m^2) is 2.5 μg (Table 1).

To get the mice exposed to similar doses of aldehydes per inhalation as those of their human counterparts smoking one cigarette, we calculated