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mRNA-induction and cytokine release during in vitro exposure of human nasal respiratory epithelia to methyl methacrylate

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Abstract

Background: Methyl methacrylate (MMA) has been reported to cause histopathological changes in rodent nasal epithelium after inhalation challenges. Data in humans are lacking.
Methods: In this in vitro design 22 primary cell cultures taken from inferior turbinate tissue of healthy individuals were exposed to MMA concentrations of 50 ppm (German MAK-value) and 200 ppm. mRNA expression and cytokine release of inflammatory mediators were quantified after 4 h and after 24 h. Controls were exposed to synthetic air. Q-PCR analysis was performed for TNF-α, IL-1β, IL-6, IL-8, MCP-1, GMCSF, Cox-1 and Cox-2. ELISA assays were performed from culture supernatants for TNF-α, IL-1β, IL-6, IL-8, MCP-1 and GMCSF.
Results: Acute inductions of mRNA after 4 h were observed for TNF-α, IL-1β, IL-6, IL-8 and MCP-1 at 50 ppm. ELISA analysis of the described parameters did not reveal any significant upregulations at both concentrations after both 4 h and 24 h.
Conclusions: The obtained data suggest that exposure of human respiratory epithelia in vitro to 50 ppm and to 200 ppm of MMA does not induce lasting upregulation of the inflammatory mediators measured in this study. The exposure limit of 50 ppm appears safe following these results obtained from human respiratory epithelia.

Keywords: Methylmethacrylate; Rhinitis; Human nasal epithelia; mRNA-induction; Cytokine release; Inflammatory mediators

1. Introduction

Methylmethacrylate (MMA) is an organic solvent widely used in various industrial processes, mostly related to the production of acrylic products like polymethyl methacrylate ("plexiglass"), paints, leather, paper or textiles. This monomer also plays a role in different medical applications and is used as a component for dental prostheses as well as for bone cement. Due to mucous membrane and eye irritation in rats at 250 ppm, the German MAK value has been set to 50 ppm. Considering this widespread use, the question whether this occupational exposure limit is adequate to prevent inflammatory disease of the upper respiratory tract in occupationally exposed individuals is highly relevant.

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Different mechanisms of reaction to airborne triggers have been described. Physical or chemical damage of the superficial epithelial cells can directly lead to a release of cytokines, which then promote the recruitment of immunocompetent cells from the blood into the tissue. Unspecific irritation can also be mediated by sensory, trigeminal nerve endings, stimulating axon reflexes and via the central nervous system causing clinical symptoms like itching, sneezing and pain (Eccles, 2000).

Solvents and aldehydes have been found to potentially cause inflammatory and degenerative mucosal changes in nasal respiratory epithelia in the animal model, but data on such effects in humans are limited. In a previous study our group showed no increase in the incidence of hyp- or anosmia in industrial workers after a median duration of exposure to MMA of 9.6 years (Muttray et al., 1997). However, in a rodent animal model long term exposure to MMA resulted in both inflammatory and histopathologic changes of respiratory mucosa (Chan et al., 1988a,b). The validity of animal models is doubtable as mucociliary transport and susceptibility to airborne toxins vary between species (Abraham et al., 1986; Cassee and Feron, 1994; Harkema et al., 1997).

In vivo studies on effects of MMA in humans are rare and in part difficult to interpret due to a lack of standardization as to the exposure to just one substance as opposed to unknown mixtures. Reports on effects on respiratory epithelia in highly polluted urban areas (Calderon-Garciduenas et al., 1994; Calderon-Garciduenas et al., 1997) as well as in occupationally exposed individuals (Dragomiretskii et al., 1970; Edling et al., 1987; Hellquist et al., 1983; Kup, 1985; Muttray et al., 2001; Welch et al., 1995) are never strictly limited to one as opposed to an unspecified mixture of hazardous substances due to the nature of these analyses. Also, studies on exposure of humans to potentially harmful airborne substances in vivo are mainly limited to low concentrations, which do not exceed the limits of the respective occupational exposure limit. In vitro models are necessary to investigate possible dose dependent effects including higher concentrations. We have developed an in vitro model, using primary cell cultures of human nasal respiratory epithelia, which has been validated in previous investigations (Gosepath et al., 2000; Riechelmann et al., 1994; Schäfer et al., 1999). This model was used in the present study, evaluating potential clinical and subclinical inflammatory effects of MMA in a dose dependant fashion. In the in vitro setting, which is presented here, we were able to compare effects of 50 ppm (MAK-value) to the four-fold concentration of 200 ppm.

2. Methods

2.1. Cell cultures

Twenty-two tissue specimens of human nasal respiratory mucosa were harvested from inferior turbinates during surgical interventions in patients without any history of inflammatory disease of the nose. Additionally, none of these individuals used any medication potentially interfering with cytokine production and release. Patients underwent submucosal turbinate resection indicated for impaired nasal breathing due to mechanical obstruction. Prior to obtaining the tissue specimens the protocol and informed consent form were approved by the institutional review boards for human beings for our institution. After mincing the specimens, tissues were digested in Protease, 0.4% (Sigma, Deisenhofen, FRG) over night at 4 °C and next day for additional 15 min at 37 °C under vigorous shaking. Single cells were isolated by passing the incubated cell suspension through a cell strainer, 70 μm pore size (Falcon, Ort) using 30 ml PBS. Cells were then collected by centrifugation (10 min, 500 g) and resuspended in 4 ml DMEM/HAM's F12 (Sigma, Deisenhofen, FRG), supplemented with 10% FCS (PAA, Cölbe, FRG) and 2% Pen/Strep (Gibco, Karlsruhe, FRG). Viability was controlled by a trypan-blue staining.

2.5×10^5 cells each were than seeded in 25 cm^2 uncoated culture flasks and cultured submerged for 24 h in 5 ml medium at 37 °C. The dominating cell types were respiratory epithelial cells (80–90%) and fibroblast cells (10–20%), defined by immunohistochemistry using an pan-Cytokeratin-monoclonal Ab (clone MNF116), and an anti-Fibroblast-monoclonal Ab (clone 5B5; both Dako, Hamburg, FRG) (data not shown). Cilliar movements were visible as a sign of viable epithelial cells. The cells were than exposed to MMA in two concentrations over 4 and 24 h. The gases with the appropriate concentrations, supplemented with 5% CO_2, were purchased from Westfalen AG, Münster, FRG. Concentrations were chosen at the level of the occupational exposure limit in Germany of 50 ppm ("MAK"-value) and at a four-fold higher level (200 ppm). Controls were exposed to synthetic air (filtered compartment air supplemented with 5% CO_2). All cultures were conducted in parallel and in duplicate for best comparability.

Four hours after the beginning of the experiments (T4) and after 24 h (T24) the respective flasks were removed from the incubators, culture media sampled and stored at −20 °C until ELISA-analysis. Cells were used for direct RNA-isolation.

2.2. Viability assay

To assess possible cytotoxic responses of the cultured cells in response to the gases, and to obtain best comparability of the data, live-dead assays were preformed before starting the exposure experiments. The live/dead-Kit was obtained from Molecular Probes, Eugene, OR, USA and used according to the manufacturer's recommendations. The assay allows