TOXIC EFFECT IN THE LUNGS OF RATS AFTER INHALATION EXPOSURE TO BENZALKONIUM CHLORIDE

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Abstract

Background: Benzalkonium chloride (BAC) is a quaternary ammonium compound (QAC) toxic to microorganisms. Inhalation is one of the major possible routes of human exposure to BAC. Materials and Methods: Experiments were performed on female Wistar rats. The rats were exposed to aerosol of BAC water solution at the target concentration of 0 (control group) and 35 mg/m 3 for 5 days (6 h/day) and, after a 2-week interval, the animals were challenged (day 21 ) with BAC aerosol at the target concentration of 0 (control group) and 35 mg/m 3 for 6 h. Results: Compared to the controls, the animals exposed to BAC aerosol were characterized by lower food intake and their body weight was significantly smaller. As regards BAC-exposed group, a significant increase was noted in relative lung mass, total protein concentration, and MIP-2 in BALF both directly after the termination of the exposure and 18 h afterwards. Significantly higher IL-6 and IgE concentrations in BALF and a decrease in the CC16 concentration in BALF were found in the exposed group immediately after the exposure. The leukocyte count in BALF was significantly higher in the animals exposed to BAC aerosol compared to the controls. In the lungs of rats exposed to BAC the following effects were observed: minimal perivascular, interstitial edema, focal aggregates of alveolar macrophages, interstitial mononuclear cell infiltrations, thickened alveolar septa and marginal lipoproteinosis. Conclusion: Inhalation of BAC induced a strong inflammatory response and a damage to the blood-air barrier. Reduced concentrations of CC16, which is an immunosuppressive and anti-inflammatory protein, in combination with increased IgE concentrations in BALF may be indicative of the immuno-inflammatory response in the animals exposed to BAC aerosol by inhalation. Histopathological examinations of tissue samples from the BAC-exposed rats revealed a number of pathological changes found only in the lungs.

Key words: Benzalkonium chloride, Rats, Inhalation, Lungs, Biomarkers, Histopathological examinations

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INTRODUCTION

Benzalkonium chloride (BAC) belongs to the group of quaternary ammonium compounds (QAC). In recent years, numerous works have been published describing the toxic activity of BAC in the in vitro and in vivo studies [1–13]. Scientific interest in BAC toxicity to the living organisms has resulted from a wide range of its use as a bactericide and preservative. It has been found that BAC used as a component of eye drops may adversely affect the different structures of the eye [1,2,5,7–10]. The widespread use of BAC also involves utilization of its bactericidal activity in the treatment of teeth [14] and the development of new nanocomposite structures with a built-in BAC molecule [15].

In some cases, the use of a BAC-containing product for its intended purpose caused a severe allergic reaction of the skin [16] and eye conjunctiva [17]. There are a number of reports of accidental exposure of humans to BAC, which led to death or a serious injury [18–20].

A major route of human exposure to this toxic compound is inhalation. Inhalation exposure of humans to preparations which include BAC results from the widespread use of BAC in various nasal sprays [21,22]. It was also found that BAC may play a role in occupational exposure [23].

In recent years, it has been demonstrated that BAC may cause an increased immune response in mice. The authors suggest that the exposure by inhalation can result in an increased risk of allergy caused by BAC [24]. In an inhalation study performed in mice, it was found that inhalation of BAC at higher concentrations induced deep lung effects and also lung inflammation [25].

In previous years we assessed toxic effects of BAC to the respiratory system and changes in the concentrations of catecholamines in the brain of rats exposed by inhalation to BAC aerosol [26,27]. The present study is an extension of our earlier works assessing the toxic effects of exposure to aerosol of BAC water solution in laboratory animals under conditions of repeated inhalation exposure.

MATERIALS AND METHODS

Animals

Female NIOM outbred Wist Wistar rats aged from 2 to 3 months obtained from the breeding farm of the Nofer Institute of Occupational Medicine, Łódź, Poland, were used in the experiment.

Chemicals

The animals were exposed in exposure chambers to Fluka BAC (CAS No. 8001-54-5, purity ≥ 95%) aerosol.

Inhalation exposure and experimental design

The chemical to be tested was injected to the exposure chamber with dynamic air flow to ensure 15 air changes per hour, where rat’s head/nose was exposed to a direct contact with the BAC aerosol. BAC was injected into the chamber in the form of aqueous aerosol. The BAC concentration in the chamber was monitored by HPLC [26,28].

The Local Ethics Committee for Experiments on Animals approved the study protocol (Opinion No. Ł/BD/247). Figure 1 is a schematic representation of the experimental protocol. Rats were exposed to BAC aerosol at the target concentration of 0 (control group) and 35 mg/m$^3$ for 5 days (6 h/day) and, after a 2-week interval, the animals were challenged (day 21) with BAC aerosol at the target concentration of 0 (control group) and 35 mg/m$^3$ for 6 h. Body weights and food intakes were measured weekly. The animals were given standard laboratory food and water ad libitum, except for the time during which they were exposed to BAC aerosol in the dynamic inhalation chambers. The chamber relative temperature and humidity were maintained at 20–22°C and 35–45%, respectively.

Biological materials were collected from the exposed and control animals at two time-points: immediately after termination of exposure (day 21) and 18 h after the BAC challenge inhalation ended (Figure 1). The rats were...