Original investigations

Ultrastructural changes in the respiratory tract of rats following methyl isocyanate inhalation

D. Dinsdale1, B. Nemery2, and S. Sparrow1

1 Toxicology Unit, MRC Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF, UK
2 Unité de Toxicologie Industrielle et Médecine du Travail, Faculté de Médecine, Université Catholique de Louvain, Bruxelles, Belgium

Abstract. The static exposure of rats to 0.25 mg/l methyl isocyanate for 1 h resulted in damage to the epithelium of the proximal bronchioles and upper airways. Bronchiolar cells exhibited both nuclear and cytoplasmic damage; many epithelial cells, particularly in the bronchi and trachea, were killed and/or dislodged from the basement membrane. A “raft” of cell debris and fibrin lined most of the airways during the 1st week after exposure but repair to the underlying epithelium was well advanced within 2–3 days. The majority of airways were lined by a normal epithelium within 3 weeks of exposure, but isolated foci of hyperplasia and occluded airways probably accounted for continued respiratory impairment.

Keywords: Lung — Methyl isocyanate — Gas poisoning — Electron microscopy — Rats

Introduction

The inhalation by rats of high but survivable doses of methyl isocyanate (MIC) results in changes in their breathing pattern which are symptomatic of a “respiratory irritant” (Nemery et al. 1985a; Ferguson et al. 1986). MIC has been shown to cause injury to the respiratory tract (Nemery et al. 1985a; Salmon et al. 1985). This injury mainly affects the airways and together with the subsequent repair process, it is the major pathological feature of MIC inhalation. An investigation of these changes was undertaken, therefore, to study the development of sequelae which may be relevant for an understanding of the long-term effects of the disaster in Bhopal (Lepkowski 1985).

Materials and methods

Five groups of six, individually caged, male rats of the LAC:P strain (130–170 g body weight) were exposed in a 50 1 sealed chamber to an initial concentration of 0.25 mg MIC/1 (100 ppm) for 1 h; the average concentration was 20–30 ppm (Nemery et al. 1985a). Three similar groups of rats were kept in the inhalation chamber, without the administration of MIC, for 1 h.

All rats were returned to their normal holding cages with free access to drinking water and MRC 41B diet. Three of the exposed animals were killed 1 h after the end of exposure. Further groups of three rats were killed 1, 2, 3, 4, 7, 14 and 21 days after exposure; pairs of controls were killed on each of these days. The remaining rats were used in another study.

All rats were anaesthetised with an intraperitoneal injection of 1 ml chloral hydrate (7%), and a cannula was inserted into the upper trachea. The diaphragm was perforated via an abdominal incision and after the resulting collapse of the lungs, they were re-inflated via the tracheal cannula with 2% glutaraldehyde in buffer 0.1 M with respect to sodium cacodylate (pH 7.3:310 mosM.kg−1) at a pressure of 250 mm water. The trachea and left lung were excised and stored overnight in the same fixative before being sub-divided for microscopy.

Transverse slices, approximately 1 mm thick, were cut from the trachea, left lung, and left bronchus for subsequent sectioning. The remainder of each trachea and bronchus was bisected longitudinally and, together with further lung slices, these samples were allocated for scanning electron microscopy.

Four slices from each lung were stained en bloc with ruthenium red (Luft 1971) and embedded in Araldite. All other samples were post-fixed in 1% osmium tetroxide in buffer (0.1 M with respect to sodium cacodylate and 0.5 M with respect to potassium ferrocyanide) for 4 h and subsequently stained en bloc in 2% uranyl acetate overnight at 60 °C. Samples for sectioning were embedded in Araldite and those for scanning electron microscopy were critical-point dried in alcohol/carbon dioxide (Dinsdale et al. 1984). All samples were examined in a JEOL 100-CX electron microscope equipped with an ASID-4D scanning unit.

Results

All rats showed signs of eye and nose irritation immediately after the introduction of MIC into the exposure chamber. A momentary apnoea was followed by a few coughs before the animals began a slow and regular but laboured breathing with gasping inspirations.

The rats displayed no signs of agitation or panic but they were clearly discomforted. A clear nasal exudate was evident within 10 min of the onset of exposure. All the rats soon settled into recumbent positions but remained conscious and moved, intermittently, to alternative positions. Following their removal from the chamber the rats continued to breathe abnormally but they soon began to walk...
Fig. 1. A Bronchial lining, 1 h after the end of exposure. A ciliated cell (c) of the epithelium, overlaid by a layer of fibrin (f) including detached epithelial cells (e). B–F Bronchiolar epithelium, 1 day after exposure. Exposed basement membrane (arrows) overlaid by a detached ciliated cell (e), a necrotic non-ciliated cell (d) and a macrophage (m). Many remaining epithelial cells have abnormal nuclei (n). A focal concentration of organelles (*) is present in the apical cytoplasm of a non-ciliated cell; ciliated crypts (open arrows) and disorganised cytoplasmic axonemes (a) are evident in a ciliated cell. All bars = 2 μm