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METHYL ISOCYANATE TOXICITY: A REVIEW OF ANIMAL EXPERIMENTAL STUDIES

2. LONG-TERM EFFECTS

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ABSTRACT

The long term effects of methyl isocyanate on various parameters of health in small animals are reviewed. The immediate changes in large animals (goats and buffaloes) are also analysed.

Introduction

Experimental studies in animals exposed to methyl isocyanate (MIC) had demonstrated that the cause of immediate death of animals was profound hypoxia that resulted from blockage of airways(1). Studies had also proved that MIC could cross blood - tissue barriers leading to multi-system toxicity. Thus, there is a possibility that MIC exposure can lead to multi-system disease in addition to respiratory effects. Therefore, this article reviews the experimental studies that have evaluated the long term effects of MIC toxicity.

In order to evaluate the long term effects of MIC, studies on experimental animals were carried out two hour after MIC inhalation exposures and during the ensuing three months. Deaths of rats and mice exposed to lethal concentrations (20 to 30 ppm) began within 15-18 hr and a second wave of deaths occurred after eight to ten days. Most deaths occurred during the first month following the exposures and were preceded by periods of severe respiratory distress. There was persistence of the increase in lung weight in animals that survived exposures to high doses of MIC and these findings suggest a proliferative response perhaps associated with a reparative process (2).

1. Respiratory effects

B6C3F1 mice when exposed by inhalation to 0,3, 10 and 30 ppm methyl isocyanate for two hours followed by a 90-day recovery period showed significant gross findings in respiratory system and in the thymus in the high dose animals (3). 20% of male mice in the 30 ppm group died following exposure. The thymus was observed to be smaller in some high dose males in days one, three and seven. Only minor changes were seen at 3 ppm. At 30 ppm, there were extensive necrosis and erosion of the respiratory and olfactory epithelium in the nasal cavity. Severe necrosis and epithelial erosions were found in trachea and bronchi. Regeneration of the mucosal epithelium occurred rapidly in the nasal cavity and airways. Intraluminal fibrotic projections covered by respiratory epithelium and bronchial fibrosis were found in the major airways of the 30 ppm male and female mice by days seven. The intraluminal fibrosis persisted to day 91. In male mice with severe bronchial fibrosis, chronic alveolitis and atelectasis were found. In mice exposed to 3 or 10 ppm, persistent pulmonary changes were not found. These studies suggest that MIC inhalation at or near lethal concentration can cause persistent fibrosis of the major bronchi in mice (3).

Bucher et al evaluated Fischer 344 rats (males and females) immediately after a single two hour exposure to 0,3,10 or 30 ppm MIC and through day 91 to study the pathology of acute inhalation exposure to MIC (4). On day one, acute inflammation and fibrino-purulent exudate partially blocked the nasal passages. Epithelial cells had sloughed from the nasopharynx, trachea, bronchi and major bronchioles, leaving the basement membrane covered with fibrin and exudate. Granulomatous inflammation and intraluminal fibrosis of the airways were observed by day three, with increased intraluminal fibrosis by day seven. Lower airways became blocked by exfoliated cells, mucous plugs, and/or intraluminal fibrosis. Damage to the lung parenchyma, even at lethal concentrations, was limited to moderate inflammation. Intraluminal fibrosis, mild bronchitis and bronchiolitis, and mucous plugs persisted throughout the 91-day study (4). Ultrastruc-

tural studies demonstrated that the respiratory and olfactory epithelium were capable of complete structural regeneration after an acute destruction by methyl isocyanate (5).

Stevens et al assessed pulmonary function in male, F344 rats one, two, three, seven, and 13 weeks after a single two hour exposure to 0,3,10 or 30 ppm methyl isocyanate (6). No significant changes were observed in the rats exposed to 3 ppm through 13 weeks. Diffusing capacity (DLCO), quasistatic lung compliance, and homogeneity of ventilation, as determined by multibreath nitrogen washout, were depressed in the rats exposed to 10 and 30 ppm by one week after exposure. None of the rats exposed to 30 ppm survived beyond one week. By 13 weeks, dramatic increases in lung volumes were observed in the rats exposed to 10 ppm, while DLCO and lung compliance were only mildly affected. However, volume-specific DLCO and compliance were depressed in the rats exposed to 10 ppm, suggesting that lung hyperinflation or other compensatory means of increasing lung size occurred in response to the methyl isocyanate-induced lung lesion. This group also exhibited increased expiratory times during tidal breathing and severely impaired distribution of vantilated air. Collectively, these results suggest the development and likely progression of a severe, obstructive airway lesion with associated gas trapping, and the existence of a pronounced concentration response relationship between 3 and 10 ppm methyl isocyanate exposure.

Cardiopulmonary function was assessed four and six months after Fischer 344 rats were exposed for two hours to 0,3, or 10 ppm methyl isocyanate (MIC) (7). During assessment, the rats were challenged with 4 and 8% carbon dioxide (CO_2) to stimulate ventilatory drive. Minute ventilation (VE) during CO_2 challenge was increased in MIC-treated rats compared to controls when examined four months after exposure to 10 ppm MIC, suggesting a ventilation / perfusion inequality. An increase in maximum expiratory flow and a decrease in expiratory time indicated increased lung recoil in these rats. Evidence of pulmonary hypertension was observed in electrocardiograms (ECG) and supported by postmortem analysis that showed a positive association between increased ECG abnormalities and increased right ventricular weights in the rats treated with 10 ppm MIC. At six months, forced expiratory flow-volume curves indicated persistent airway obstruction; however, no changes in inspiratory or expiratory resistance were evident. Decreased dynamic compliance and changes in two measures of lung function (volume and time at zero expiratory intrapleural pressure) suggested that MIC induced lung dysfunction also exhibited elements of a restrictive disease(7).

2. Eye changes

The release of methyl isocyanate gas in Bhopal, was reported to cause temporary blindness and other eye injuries in many of the exposed people. Methyl isocyanate (MIC) is known to be corrosive and to irritate intact skin and mucous membranes, but little is known about the extent of ocular damage incurred during exposure to its vapors. The eyes of male and female Fischer 344 rats were evaluated immediately after a two hour exposure to 0,3,10 or 30 ppm of MIC, and periodically thereafter during a 91 -day recovery period (8). During exposure to 10 ppm and higher concentration, rats kept their eyes partially closed, Copious lacrimation and occasional frothy nasal discharge were evident. Eyes were examined under ultraviolet light after topical application of sodium fluorescein, and histopathologic examination included lids, cornea, lens, retina, optic nerve, and Harderian gland. There was no significant gross or microscopic evidence of epithelial erosion or ulceration of the cornea, or of adjacent tissues immediately after, or at any time following exposure(8). Exposure to these concentrations had resulted in severe necrotizing effect on the olfactory and respiratory epithelium lining the respiratory tract, but no effect was observed in those areas of the nose lined with stratified squamous epithelium (4). Stratified squamous epithelium also covers the anterior portion of the cornea and the apparent lower sensitivity of this cell type to MIC-induced damage may account for the lack of corneal injury (8). Natural protective mechanisms of the eye that include extensive tearing and eye lid closure might have also minimized direct contact of the irritating vapour to the eye(8).

3. Immunotoxicity

The immunotoxicity of MIC was evaluated in female B6C3E1 mice exposed via inhalation to 0, 1 or 3 ppm for six hours per day on four consecutive days. The antibody response to sheep erythrocytes and natural killer cell activity were found to be unafffected by MIC exposure. Although lymphoproliferative responses to mitogen were moderately suppresed by MIC, the differences were not statistically significant. The response of splenic lymphocytes to allogenic leukocytes in a mixed leukocyte response (MLR) was suppressed in a dose-related fashion and

was significantly different from the control response at 3 ppm level. This effect was thought to be secondary and a result of general toxicity, rather than a direct effect of MIC on the immune system. Furthermore, resistance to the infectious agents Listeria monocytogenes, mouse malaria parasite and influenza virus or to transplantable tumor cells was not compromised by MIC exposure. Thus, the immune systems do not appear to be a primary target for MIC toxicity (9). In order to evaluate antibody response to MIC exposure, guinea pigs were injected with MIC in its reactive isocyanate form (10). Three weeks later, blood was drawn and serum evaluated using ELISA. To detect antibodies, an antigen was prepared by reaction of MIC with guinea pig serum albumin. Antibodies were detected in each of the four animals injected with MIC. Titres achieved were 1:5120 to 1:10240. Inhibition assays revealed antibody specificity directed towards the MIC hapten (10).

4. Reproduction

Inhaled MIC at concentration of 0,1, or 3 ppm, six hours per day during days 14 through 17 of gestation caused a significant increase in the number of dead fetuses at birth and caused a significant decrease in neonatal survival during lactation. In contrast, exposure of male and female mice to 1 or 3 ppm given six hours per day for four consecutive days had no effect on reproduction during mating trials conducted one, eight and 17 weeks after the exposure period. Similarly, no dominant lethal effect was observed in exposed male mated to untreated female mice, suggesting that exposure of a conceptus in utero results in more toxicity than exposure of the gonadal cells prior to mating (11). It had also been shown that exposure of mice to relatively low concentrations of MIC (9 and 15 ppm) for three hours had resulted in loss of all foetuses in 75% of animals (12). Methyl isocyanate forms a labile conjugate, S-(N-methyl carbamoyl) GSH(SMG) by way of a reversible reaction with GSH (reduced glutathione). The toxicity of SMG was studied (13) on mouse embryos on day eight of gestation and cultured in serum for 42 hr., SMG caused concentration-dependent decrease in growth and development over the range of 0.1mM-2mM without causing significant mortality. At a concentration of 0.25mM, spinal kinks and somite pair distortion in the region of the forelimb were evident in 38% of embryos. It is concluded that SMG exerts embryotoxic and dysmorphogenic effects and may contribute to systemic toxicity of methyl isocyanate(13).

5. Genetic damage

To assess the possibility that MIC exposure might lead to genetic damage, MIC was tested in a number of in vitro and in vivo assays. MIC gave negative results in the Ames test and in the Drosophila sex-linked recessive lethal test. However, in cultured mammalian cells, positive results were obtained in the mouse lymphoma TK +/- assay, and both sister chromatid exchanges and chromosomal aberrations were increased in chinese hamster ovary cells. In in vivo assessments, small increases in sister chromatid exchanges occurred in lung cells, but not in peripheral blood lymphocytes (14). The most consistent finding in in vivo genetic toxicity studies was a profound delay in the cell cycle time of bone marrow cells (14,15) and was in agreement with the findings of Hong et al (16). From these results and from consideration of the chemistry of isoyanate-DNA and isocyanate-protein reactions, it was speculated that MIC may exert its genotoxic activity through interactions with nuclear proteins affecting chromosomal structure rather than through direct genetic mutations(14).

6. Effects of exposure on Livestocks

Animals(buffaloes,goats) died within two to three minutes of inhaling the gas and had the symptoms of frothy discharge from the mouth, lacrimation, acute dyspnoea, open mouth breathing, abortions mostly in late stages, circling and death(18). Surviving animals were dyspnoeic with open mouth breathing and had dry muzzle, red conjunctivae and abdominal respiration. There was accumulation of fluid in the lower thoracic cavity with muffling of heart sounds. There was immediate drying up of milk after exposure and milk production came down from about eight to 10 Kg per day to half Kg to nil. Low levels of methylamine and dimethyl urea residues were found in milk and meat samples. Milk and meat of the gas exposed animals fed to laboratory animals did not cause any adverse effect. Serum and urine thiocyanate levels were found to be higher in affected animals and declined after three months. Varying degrees of interstitial fibrosis with right heart enlargement were seen on radiographic examination. Arterial PO_2 was low with normal $PaCO_2$. Histopathological studies revealed mild to moderate perivascular cuffing of lymphocytes, satellitosis and neuronophagia in brain. Lungs showed thickened interalveolar septa and narrowing of alveolar air spaces. In kids, there were thickening of pleura associated with fibrosis and focal to diffuse proliferation of the lining epithelial cells in bronchi and bronchioles. Other organs such as heart, liver

and kidneys showed changes varying from cloudy swelling to fatty changes and even necrosis (18).

The above mentioned animal experimental studies are related to toxicity of methyl isocyanate. These results cannot be directly extrapolated to the situation at Bhopl. Even though MIC is the primary chemical released from the tank No. 610 on 2/3rd December 1984, the effects of several reaction products following the incident including hydrogen cyanide on the human health requires further in-depth study. However, it may not be technically feasible to recreate the accident experimentally in order to examine the effects in animals(17). Even though the experimental studies provide an insight into the human health effects that may result from the disaster, the real picture regarding the effects of the toxic gas on human beings will be available only from the studies carried out in the victims at Bhopal.

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