NON-SPECIFIC CARDIOVASCULAR DEPRESSANT EFFECT OF METHYL ISOCYANATE (MIC) IN RATS

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Accepted March 6, 1989

Abstract: Methyl isocyanate (MIC) either inhaled (5, 10 mg/lit) or administered by intravenous (5, 10, 28 mg/kg) or subcutaneous (1300, 1500 mg/kg) routes produced a dose dependent fall in blood pressure (BP) and heart rate (HR) in anaesthetised rats. Higher doses (10 mg/lit inhalation, 10 & 28 mg/kg i. v., 1500 mg/kg s. c.) increased the lung body weight index (LBI) and tracheobronchial resistance (TBR) concomitant with gross pulmonary damage and edema. However, lower doses (5 mg/lit inhalation, 5 mg/kg i. v., 1300 mg/kg s. c.) produced the cardiovascular depressant effect without affecting LBI, lung morphology and TBR. The effects of MIC on BP, HR and TBR were not counteracted by muscarinic, histaminic and 5-HT receptor blockers and by vagotomy. Studies with hydrolysis products of MIC showed that relatively large doses of methylamine (MA) and dimethylurea (DMU) (i. v.) produced cardiovascular depressant effects, without affecting the LBI & TBR. The results indicate that the cardiovascular depressant effect of MIC may not be entirely a sequel to its effect on respiratory organs, release of vasoactive substances or its hydrolysis products. A non-specific cardiovascular depressant effect of MIC is suggested.

Key words: Methylisocyanate, cardiovascular depression.

INTRODUCTION

Eversince Kimmerle and Eben (1964) reported the toxicity of methyl isocyanate (MIC) as a primarily irritant of respiratory organs with its dominant action on the mucous membranes, subsequent studies were limited to assess the damage to respiratory organs caused by MIC (Salmon et al., 1985; Nemery et al., 1985; Dodd et al., 1986; Dodd and Fowler, 1986; Fowler and Dodd, 1986; Boorman et al.,

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(1987). As a consequence little information is available on the effects of MIC on other vital organ systems of the body. The other factor that seems to have distracted the investigators is the report that MIC reacts with decomposition in water (Kimmerle and Eben, 1964), implying thereby that MIC on entering the body, will be hydrolysed by the body fluids and lose its biological activity. However, recent reports that MIC is fairly stable in water (Ferguson et al., 1986; Meshram and Rao, 1988) evoked renewed interest in the toxicity of MIC on organs other than the lungs. The present study was designed to evaluate the cardiovascular effects of MIC independent of its effects on pulmonary sites.

MATERIALS AND METHODS

Animals

Forty male Wistar rats weighing 170–210 g obtained from the animal house of the Establishment and fed on standard Hind Lear feed were used in this study.

PHYSIOLOGICAL PARAMETERS:

Rats were prepared under pentobarbital sodium (40 mg/kg i. p.) anaesthesia for recording various physiological parameters on a Grass Polygraph Model 7. All animals, except those used in inhalation study were maintained on positive pressure ventilation using rodent ventilator (Ugo Basile). A bronchospasm transducer (7020 Ugo Basile) was used to measure tracheobronchial resistance. The mean carotid blood pressure (BP) was recorded using a P23Dc Statham Transducer. The signals from DC Driver amplifier recording BP were fed into the Tachograph preamplifier (7P4G) to record heart rate. Continuous monitoring of EKG (Lead II) was done using EKG–Tachograph preamplifier (7P4G) and displayed on Oscilloscope. Tidal volume was recorded using Pneumotachograph (Fleisch Tube 0000) and the low pressure differential transducer (SWEMA SP 20400). The tidal volume signals thus generated were fed to the Polygraph integrator (7P10B) to record the minute volume.

ASSESSMENT OF LUNG DAMAGE:

Gross pulmonary damage and edema were assessed in excised lungs of rats treated with MIC (inhalation and i. v.) immediately after the termination of studies on physiological parameters.

(i) Lung-Body weight index (LBI): Lungs were dissected out and were freed from adhering blood and extraneous tissues and weighed. The LBI was calculated by the formula:

\[ \text{LBI} = \frac{\text{Lung (Wet) Weight}}{\text{Body Weight}} \times 100 \]

(ii) Histopathological studies: Paraffin embedded lung tissue was sliced into 5–6 μ thick sections, stained with haematoxyline and eosin and were examined under light microscope.

DRUG TREATMENT:

MIC was administered by inhalation, intravenous and subcutaneous routes. For
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Inhalation, desired amount of MIC was spontaneously evaporated in a 1 lit all glass bottle fitted with a stainless steel outlet which was connected temporarily to the tracheal cannula. Intravenous administration of MIC and other drugs was done through an indwelling tube in the jugular vein.

MIC was freshly diluted in chilled normal saline. Other drugs were diluted in normal saline at room temperature.

Route of administration, dose and time of administration of blockers (mentioned under results) were the same as mentioned in the literature (Ghosh, 1984).

Doses of methylamine (MA) and dimethylurea (DMU) were arrived at on the basis of hydrolysis products of MIC. Thus one mole of MIC (57 g) will produce on hydrolysis 1 mole of MA (31 g). One mole of MA will further react with 1 mole of MIC to give one mole of DMU (88 g).

\[
\text{CH}_3\text{NCO} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{NH}_2 + \text{CO}_2 \\
\text{CH}_3\text{NCO} + \text{CH}_3\text{NH}_2 \rightarrow \text{CH}_3\text{NHCONHCH}_3
\]

Moreover, since chloride salt of MA has been used in the present study appropriate corrections were made to arrive at the quantity of MA.

**CHEMICALS & DRUGS**

Methyl isocyanate (99 % pure) was synthesized in the chemical laboratories of this Establishment (Kaushik et al., 1987). Atropine (Bengal Immunity), Cimetidine (Franco Indian Biologicals), Cyproheptadine hydrochloride (Merck Sharp and Dohme), Promethazine HCl (May and Baker), Methylamine hydrochloride (Sigma), and N, N’dimethyl urea (Fluka AG) were procured from the trade.

**RESULTS**

**EFFECT ON BLOOD PRESSURE AND HEART RATE**

(i) **Inhalation**

Inhalation of MIC (5 mg/lit) for 3 minutes produced reversible short lived fall in blood pressure (BP) concomitant with a marked drop in heart rate (HR). However, the effect on respiratory rate was negligible. Breathing of room air from the same restricted source (1 lit bottle) had no effect on any of the parameters studied (Fig. 1). Higher concentration of MIC (10 mg/lit) resulted in an abrupt and very pronounced fall in BP, HR and respiratory rate culminating in the death of the animals.

(ii) **Intravenous Administration**

Essentially, similar effects on BP and HR were produced by MIC administered intravenously in doses of 5, 10 and 28 mg/kg. However, the tracheo bronchial resistance (TBR) tracings showed severe obstruction in the airways appearing 90 sec after MIC (10 and 28 mg/kg) administration (Fig. 2, 3). An interesting observation in this series of experiments was that MIC at a dose level of 5 mg/kg produced initial pronounced fall in BP and HR which returned to normal levels in 2–3 min. MIC at this dose level had no effect on the TBR (Fig. 2). It may be noted that a few spikes
Fig. 1. Inhalation of MIC (5 mg/l for 3 min) evoked short lived fall in BP and HR. There was no marked effect on respiratory rate.

Fig. 2. Lower dose of MIC (5 mg/kg i. v.) produced reversible fall in BP and HR. It may be noted that the TBR was not affected. However, higher dose (10 mg/kg i. v.) produced a very marked and irreversible fall in BP concomitant with increased TBR. The animal died in 6 min.
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Fig. 3. Intravenous administration of a massive dose of MIC (28 mg/kg) produced severe TBR obstruction, fall in BP and HR. The effect on TBR became more marked after 3 min.

appeared in the TBR tracings immediately after the completion of i. v. injection of MIC (Fig. 2, 3). These coincided with writhing observed in the animals.

(iii) Subcutaneous Administration of MIC

Administration of MIC (1300 mg/kg = 4 LD₅₀) by subcutaneous route produced immediate but sustained hypotension and decrease in HR. The most relevant finding was that the effect on tidal volume (TV) and minute volume (MV) appeared after a lapse of 3 minutes (Fig. 4). The animal died after about 2 hours. EKG (Lead II) displayed on oscilloscope following subcutaneous administration of MIC 1300 mg/kg, showed depression or even absence of 'P' wave. Higher doses (1500 mg and above) produced gross abnormalities in the EKG. The time of occurrence of sinus arrest (absence of 'P' wave) corresponds to the time when BP and HR are markedly low but TV and MV were not affected.

EFFECTS OF VAGOTOMY AND MUSCARINIC, HISTAMINIC, 5-HT, RECEPTORS BLOCKERS ON MIC INDUCED CARDIOVASCULAR DEPRESSION:

In separate experiments, bilateral vagotomy and pretreatment with blockers of muscarinic (Atropine, 1 mg/kg i. v.) histaminic (Cimetidine, 100 mg/kg, Promethazine, 5 mg/kg i. p.) and 5-HT (Cyprokeptadine, 50 µg/kg s. c.) receptors, failed to block the cardiovascular depressant effects of MIC administered intravenously (5 and 10 mg/kg) or inhaled (10 mg/l).
Fig. 4. Subcutaneous administration of MIC (4 LD_{50}) produced sustained fall in BP & HR. The depressant effect on tidal volume (TV) and minute volume (MV) however appeared much later. Last panel shows partial and temporary recovery by saline infusion. However, the animal died subsequently.

**EFFECTS OF METHYLAMINE AND DIMETHYLUREA ON TRACHEO-BRONCHIAL RESISTANCE, BLOOD PRESSURE, HEART RATE AND LUNG BODY WEIGHT INDEX:**

(i) Effects of methylamine (MA): Intravenous administration of methylamine hydrochloride (3, 10 and 26 mg/kg in terms of methylamine) produced dose-dependent hypotension and bradycardia. Both these effects were reversible and lasted for 2-15 min depending on the dose employed. The pattern of hypotensive response was dissimilar to that obtained with MIC in that the downward peak denoting abrupt fall in BP and bronchial resistance were absent (Fig. 5).

(ii) Effect of dimethylurea (DMU): Dimethylurea administered intravenously in doses of 5, 10 and 20 mg/kg was found to be devoid of any effect on the BP and HR. However, a dose of 40 mg/kg of DMU (equivalent to equimolecular weight of 46.48 mg/kg of MIC) produced gradual and sustained fall in BP which could be temporarily reversed by infusion of normal saline. Decrement in HR occurred after 45 min and coincided with death of the animals.

**ASSESSMENT OF EFFECTS OF MIC ON LUNGS:**

(i) Lung-body weight index (LBI): LBI of randomly picked up rats treated with MIC by different routes and rats injected with methylamine (26.0 mg/kg i.v.) was determined. There was a marked increase in the LBI of rats from 0.62±0.03 (untreated) to 1.06±0.20 and more that received higher doses of MIC (10 mg/kg i.v., 10 mg/lit inhalation). Methylamine (26.0 mg/kg i.v.) had no effect on LBI. The
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**Fig. 5** Cardiovascular depressant effect of methylamine HCl (26 mg/kg i. v.). Effects on BP and HR were reversible. The TBR remained unaffected.

most interesting finding was that MIC in lower doses 5 mg/kg i.v. or 5 mg/lit for 3 min by inhalation did not affect the LBI, although varying degree of hypotension was produced in all the rats.

(ii) Histopathological studies: Varying degree of gross pulmonary damage, edema and inflammatory reaction of the alveolar septa were the most common histopathological observations in the lungs of rats that either inhaled or received MIC by intravenous route. Inhalation of higher concentration of MIC (10 mg.l⁻¹) caused pronounced pulmonary edema and inflammatory reaction (Photo. 1a). However, gross and histological pulmonary pathology was less marked with lower concentration of MIC (Photo. 1b). Genesis of pulmonary edema with intravenously administered MIC (10 mg/kg and above) was however, the most marked effect on lungs (Photo. 1c). It is of interest to note that the cardiovascular depressant effect of MIC was not attended by gross pulmonary damage and edema at low dose levels.

**DISCUSSION**

MIC either inhaled or administered by systemic routes produced a dose-dependent depressant effect on BP & HR. Furthermore, the cardiovascular depressant effect was not due to the hydrolysis products of MIC such as MA and DMU. These findings substantiated by the reported observation that MIC remains stable in water for a few minutes (Fergusson et al., 1986; Meshram and Rao, 1988) are suggestive of the fact that MIC could exert its biological effects before it is rendered ineffective by the process of hydrolysis by body fluids.

Since MIC is classified as a primarily lung damaging agent (Fowler and Dodd,
Photo. 1. Photomicrograph (H & E Stain : × 64) of effect of MIC on lung histomorphology. MIC (10 mg/l inhaled) produced severe inflammatory reaction and pulmonary edema (la) but the lung damage was negligible with lower concentration of inhaled MIC (lb). MIC (10 mg/kg i. v.) however produced severe pulmonary edema (lc).
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1986; Boorman et al., 1987) and is known to cause impairment of respiration by a reflex inhibition (Nemery et al., 1985; Fergusson et al., 1986), one would be inclined to believe that haemodynamic effects of MIC may be a sequel to its effect on respiration. Our findings, however, show unequivocally that the cardiovascular depressant effect of MIC at low doses either inhaled or injected intravenously/subcutaneously was seen in the absence of its effect on respiratory organs as adjudged by LBI, histopathological studies of lung tissue, respiratory rate, tidal volume, minute volume and tracheobronchial resistance. No doubt, higher doses produced both the cardiovascular and respiratory changes making it difficult to discern the cardiovascular effects from its respiratory component.

MIC is reported to cause acid burns and severe tissue necrosis (Kimmerle and Eben, 1964) and since chemicals causing gross cell damage are often implicated with the release of endogenous substances resulting in severe hypotension and shock (Douglas, 1980), the possibility of release of hypotensive substance by MIC, was also examined. However, inability of various blockers to counteract the cardiovascular depressant effect of MIC exclude the involvement of any of the investigated cardiovascular depressant endogenous substances that could have been released by MIC. It is thus reasonable to suggest that the cardiovascular depressant effect of MIC is evoked by a non-specific mechanism.

ACKNOWLEDGEMENTS

The authors are thankful to Brig. K. M. Rao, Director and Dr. P. K. Ramachandran, Ex. Director, Defence Research & Development Establishment, Gwalior for their keen interest in the work and to Mr. Hari Afley for secretarial assistance. One of the authors R. K. Srivastava acknowledges with thanks the financial assistance provided by CSIR (India).

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