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Modulation of Microsomal Membrane Associated Detoxication Enzymes Activity by Methyl Isocyanate (MIC) Exposure

Anil Mishra,* Premendra D. Dwivedi, Ashish S. Verma, Jaya Mishra, M. Sinha, K. K. Dutta, and P. K. Ray

Inhalation Toxicology Division, Industrial Toxicology Research Centre, Mahatma Gandhi Marg, Post Box 80, Lucknow-226 001, India

In the industrial disaster at Bhopal, India in 1984 the leakage of 40 tons of methyl isocyanate (MIC) gas from a pesticide plant claimed over 2500 lives and left an estimated population of 15,000 people suffering from a variety of ailments (Anderson et al. 1988). Dutta et al. (1988), reported that MIC induced changes in respiratory functions including bronchial asthma, pulmonary oedema, necrosis of alveoli, hypersensitivity, pneumonities and incapacitated pulmonary functions. Reports have also indicated changes in the respiratory tract of animals as a result of exposure to MIC, the lung being the main target organ attacked by MIC (Varma et al. 1983; Dinsdale et al. 1987; Boorman et al. 1987). Biochemical studies with isocyanates have revealed that primary reactions of isocyanates with biological entities, like carbamylation of hemoglobin S (HbS) at alpha amino groups in the cells do take place (Cerami et al. 1971 ; Lee 1976). Bucher (1987) observed an increase in blood latic acid accompanied by a drop in pH in MIC exposed guinea pigs. Our earlier report of the high dose single exposure toxicity to methyl isocyanate leads to alterations in biotransformation enzymes and their isoenzymic pattern (Mishra et al.1988a ; Gupta et al. 1988). Recently, Agarwal et al. (1990) has shown a drastic decrease in Na^+, K^+ and Mg^{++}ATPase enzyme levels by repeated MIC exposure. The present study further explores the biochemical basis of MIC intoxication with reference to consecutive dose dependent toxicity in terms of alteration in phase I and phase II membrane bound detoxication enzymes.

MATERIALS AND METHODS

Adult healthy male albino rats (150 $\pm$ 10 gm body weight) of ITRC Gheru campus animal house colony, were used for this study. All the general chemicals were purchased from Sisco Research Laboratory, (India) and substrates for enzyme estimation was procured from Sigma (USA). Methyl isocyanate (MIC) was synthesized in our laboratory by the method of Mironov et al. (1969).

(*) Corresponding author

Male albino rats (150 ± 10 gm) were randomly grouped and exposed to different concentration of MIC vapours for 8 minutes during each exposure in static condition in all glass whole body inhalation chamber according to the method of Dutta et al. (1988). The treatment schedule for control and experimental animals has been described in Table 1.

Table 1. Animal treatment schedule.

Group	Number of animals	Dose and Schedule of MIC exposur	Schedule of sacrifice
I	6	355 ppm* (800 mg/m3) single exposure	All animals 7 days, after exposure
II	6	355 ppm* (800 mg/m3) two exposure at 7 days interval	All animals 7 days, after last exposure
III	6	355 ppm* (800 mg/m3) four exposure at 7 days interval	All animals 7 days, after last exposure
IV	40	1420 ppm* (3200 mg/m3) single exposure	Six animals each at 0, 24, 72, 90 hrs. and 7, 14 days after exposure
V	20	355ppm* (800 mg/m3) exposure for four consecutive days	6 animals each at 7, 14, and 21 days after last exposure
VI	75	exposure with compressed air to serve as control	Six animals with each experimental group

* Dose selected according to Agarwal et al. (1990)

At the end of each treatment schedule the six animals each from control and treated groups were sacrificed and 10% (w/v) homogenate of each lung was prepared in chilled 0.1 M phosphate buffer, pH 7.4 using a Potter Elvehjem Homogenizer with ten up and down strokes. The respective homogenates were subjected to subcellular fractionation using separation procedure as described earlier (Mishra et al. 1988a). The microsomal fractions were used for the estimation of enzyme activities.

Aniline hydroxylase (AH) activity, using aniline hydrochloride as substrate was assayed according to the procedure of Mazel (1971) and the specific activity was expressed as n moles p- aminophenol formed/mg protein/10 min.

Aminopyrine-N-demethylase (APD) activity, using 4- aminoantipyrine as substrate was assayed according to Mazel (1971) and specific activity was expressed as n moles HCHO formed/mg protein/10 min.

Glutathione-S-transferase activity, using 1-chloro, 2, 4-dinitrobenzene (CDNB) as substract was assayed by the method of Habig et al. (1974) and specific activity was expressed an μ moles conjugate formed/10min/mg protein using a molar extinction coefficient of 9.6.

The contents of reduced glutathione (GSH) and total sulphydryl (T-SH) were assayed according to the method of Jollow et al. (1974) and reported in terms of μg SH/gm tissue. Protein content were measured by the method of Lowry et al. (1951) using BSA as a standard.

RESULTS AND DISCUSSION

Activities of some of the membrane bound detoxication enzymes were estimated in the microsomes of lung in the group of animals receiving single exposure of low dose 355 ppm MIC; high dose 1420 ppm MIC and repeated low dose exposure 355 ppm MIC as per schedule. The data has been summarized in tables 2-4.

With the low dose single exposure Group I after seven days, the activities of different enzymes viz. glutathione-S-transferase, aniline hydroxylase and aminopyrine demethylase in the lungs showed non significant changes including sulphydryl contents (TSH) (Table 2). But a significant decrease in the

Table 2. Effect of repeated exposures of 355 ppm MIC on some phase I & II detoxication enzyme activity and bio-constituents of lungs

		Glutathione contents (a)	Total Sulphydryl contents (b)	Glutathione-s-transferase activity (c)	Aniline hydroxylase activity (d)	Amino pyrene demethylase activity (e)
Group I	C	3.08 ± 0.13	12.06 ± 0.93	118.3 ± 9.06	23.09 ± 2.1	13.98 ± 1.8
	E	3.23 ± 0.09	10.70 ± 0.31	132.6 ± 6.12	24.96 ± 1.43	15.18 ± 1.3
Group II	C	2.96 ± 0.48	11.48 ± 0.71	113.6 ± 7.2	21.32 ± 1.7	14.65 ± 0.98
	E	1.73 ± 0.37**	9.06 ± 1.03	146.4 ± 3.18**	15.31 ± 0.96*	10.16 ± 1.08*
Group III	C	2.83 ± 0.26	11.93 ± 0.50	121.71 ± 3.86	22.18 ± 1.32	12.86 ± 0.43
	E	1.63 ± 0.15**	7.36 ± 0.94**	173.37 ± 1.19**	13.18 ± 0.71**	10.91 ± 0.38*

All values are mean ± SE of six animals; * $p < 0.05$; ** $p < 0.001$; a, b = μg -SH/gm tissue; c = μ mole conjugate formed/mg protein/10 min.; d = n mole p-aminophenol formed / mg protein /10 min; e = n mole HCHO formed / mg protein /10 min; C = Control ; E = Experimental

Table 3. Effect of a single exposure of MIC 1420 ppm on some phase I & II detoxicatioon enzymes activities and bioconstituents of lungs (Group IV)

	Glutathione contents (a)	Total Sulphy dryl contents (b)	Glutathione-s transferase activity (c)	Aniline hydroxy-lase activity (d)	Aminopyrene demethylase activity (e)
Control	3.96 ± 0.33	20.29 ± 2.36	108.86 ± 3.73	21.17 ± 0.36	14.71 ± 0.89
Immediate	2.63 ± 0.31*	15.13 ± 1.93*	123.32 ± 2.89*	16.15 ± 0.18*	11.44 ± 0.76*
24 hrs	2.16 ± 0.37*	14.94 ± 2.01*	136.73 ± 3.06*	17.8 ± 0.17*	12.49 ± 0.93*
72 hrs	2.46 ± 0.61*	15.47 ± 1.83*	138.43 ± 3.98*	16.5 ± 0.23*	12.77 ± 0.82*
90 hrs	2.41 ± 0.43*	15.73 ± 0.93*	151.37 ± 3.08*	16.6 ± 0.47*	11.91 ± 1.10
7 days	2.48 ± 0.21*	17.76 ± 2.81*	132.36 ± 1.32*	17.8 ± 0.34*	11.68 ± 1.17
14 days	2.93 ± 0.17*	17.38 ± 1.96*	137.18 ± 2.09*	19.6 ± 1.96	12.98 ± 1.37

All values are mean ± SE of six animals; * $p < 0.05$; a, b = µg - SH/gm tissue; c = µ mole conjugate formed/mg protein/10 min.; d = n mole p- aminophenol formed/mg protein /10 min; e = n mole HCHO formed / mg protein / 10 min.

Table 4. Effect of 355 ppm of MIC /day for four consecutive days on some phase I & II detoxication enzyme levels and bioconstituents of lung (Group V)

	Glutathione contents (a)	Total sulphy dryl contents (b)	Glutathione-s transferase activity (c)	Aniline hydroxylase activity (d)	Aminopyrene demethylase activity (e)
Control	2.98 ± 0.63	12.00 ± 0.33	128.32 ± 3.96	20.93 ± 2.5	13.98 ± 0.83
7 days	1.31 ± 0.41**	8.39 ± 0.71**	183.18 ± 4.12**	14.33 ± 1.10**	8.71 ± 0.44**
14 days	1.58 ± 0.51*	8.86 ± 0.57**	181.0 ± 3.08**	13.85 ± 0.91**	9.42 ± 0.75*
21 days	1.86 ± 0.23*	10.49 ± 1.26	167.46 ± 1.96*	14.78 ± 0.83*	10.74 ± 0.66

All values are mean ± SE of six animals; * $p < 0.05$; ** $p < 0.001$
a, b = µg -SH/gm tissue; c = µ mole conjugate formed/mg protein/10min.; d = n mole p-aminophenol formed/mg protein/10 min; e = n mole HCHO formed / mg protein / 10 min.

glutathione content with a marked increase in glutathone-S-transferase activity was observed in group II including a significant decrease in the activities of aniline hydroxylase, aminopyrine demethylase total and non-protein sulphydryl contents (Table 2). In the animals of group III lung after seven days of exposure showed a highly significant increase in the glutathione-S- transferase activity with a decrease in the activities of aniline hydroxylase, aminopyrine demethylase and glutathione and total sulphydryl contents (Table 2).

Group IV also, showed a decrease in the activities of aniline hydroxylasee, aminopyrine demethylase, total and non protein sulphydryl content while an increase in the activities of glutathione-S-transferase during different post exposure periods (Table 3). The changes are statistically significant ($p<0.05$) and detailed results are summarized in Table 3.

Animals of Group V at 7 and 14 days post exposure showed a highly significant decrease in the activities of aniline hydroxylase, aminopyrine demethylase, glutathione and total sulphydryl content while a significant increase in the glutathione-S- transferase activity. The animals after twenty one days of exposure showed a little recovery in the phase I and II detoxication enzyme activities (Table 4).

Bend et al. (1985) showed that the lung tissues has an active xenobiotic metabolising capacity. Various xenobiotic exposure through inhalation has been found to affect the activities of drug metabolizing enzymes primarily (Mishra et al. 1988b; Greim 1981 ; Mannering 1971). Some studies also showed alterations in mixed function oxidase system of lung on metabolism of polycyclic aromatic hydrocarbons, furans, carbon tetrachloride, furfural and bromobenzene (Mishra et al. 1988c; Smith et al. 1986). Though several reports on the toxicity of MIC in experimental animals are available, the biochemical basis of MIC toxicity is not understood. Alteration in the activities of pulmonary mixed function oxidase system due to MIC shows that lung tissue has been the primary target organ affected by exposure to MIC. Extensive pathlogical reports after exposure to lethal concentrations of MIC showed the loss of epithelial cells and alveolar damage in the lungs (Dutta et al. 1988). Our present findings of detoxication enzymes in MIC exposed animals substantiate these reports. The catalytic and redox constituents associated with the microsomal membrane in a variety of tissues are known for their key role in metabolic disposition of xenobiotics. Their response to MIC exposure and possible contribution in its detoxication in rat lung subsequent to a single dose of 1420 ppm MIC or four consecutive doses of 355 ppm MIC resulted in a highly significant decrease in the activities of aniline hydroxylase and aminopyrine demethylase along with a marked depletion of glutathione contents and substantially elevated the glutathione-S-transferase. Pearson et al. (1990) reported that MIC forms a chemically reactive glutathione conjugate S-(N- methylcaramoyl) glutathione, a metabolite isolated from the bile of rats administered MIC. The formation of this metabolite

may be a major factor behind the reduction in the glutathione contents of our present study. The observation of Srivastava et al. (1988) regarding the reduction of blood glutathione levels in MIC exposed population of Bhopal corborate our finding of reduction in the sulphydryl compounds in MIC exposed rat lungs. All these observations indicate the toxic stress by MIC may be targeted to the microsomal membrane and that the magnitude of such stress is more on repeated exposure, possibly due to morphological injury and slower tissue regeneration as compared to a single exposure. It must be stressed here that cumulative concentrations of lower doses in repetative exposure (355 ppm) of MIC was equivalent to higher concentration single exposure of MIC (1420 ppm). However, much more detailed studies are needed to understand the exact mechanism of MIC toxication and detoxication.

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