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Effect of Methacrylonitrile on Rat Lung Antioxidant Enzymes

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Methacrylonitrile (MeAN) belongs to the family of aliphatic nitriles which, despite structural similarities with Acrylonitrile, Malononitrile exhibits entirely different biochemical effects of Adrenal necrosis (Szabo *et al* 1980), nuclear changes in neurons and Satellite ganglia (VanBreeman and Hirakoa 1961). It is used as a monomer in the production of plastic elastomers and coatings (Windholz 1983; Sax and Lewis 1987) and as a replacement for acrylonitrile in the manufacture of carbonated beverage containers (Cosidine 1974). MeAN has been identified as a component of the mainstream smoke of unfiltered cigarettes made from air-cured or flue-cured or a blend of these tobaccos (Baker *et al.* 1984). Hence, there is a large human population at risk either through direct occupational exposure or ambient environmental exposure or from contact with products containing MeAN. MeAN has been reported to be highly toxic in mice, rats, and rabbits by dermal, respiratory, and oral routes of administration (McOmie 1949; Smyth *et al.* 1962).

Despite its considerable industrial use, the literature on the mechanism of MeAN toxicity is limited. MeAN-derived radioactivity is reported to accumulate in lungs (Cavazos *et al.* 1989). MeAN depletes glutathione, both *in vivo* and *in vitro* (Day *et al.* 1988). The present study had therefore been carried out to the influence of MeAN on lipid peroxide and assess to determine the status of antioxidant enzymes in the rat lung.

Glutathione, a pulmanary cytosolic tripepeptide postulated to be an important component of the defense against oxidative injury in the lung, plays an important role in the MeAN detoxification mechanism. MeAN undergoes conjugation with reduced glutathione which could be catalysed by glutathione-S-transferase and/or proceed nonenzymatically (Silver *et al.*, 1982; Ghanayem *et al.*, 1994). An earlier study also reported the depletion of reduced glutathione by MeAN in vivo (Day *et al.*, 1998) and in vitro (Meister 1988). The depletion of GSH could lead to an impairment in the defense against oxidative injury to the lung.

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MATERIALS AND METHODS

(MeAN) was purchased from Fluka Chemicals, Switzerland. Male albino Wistar rats weighing 100-120g were purchased from the Fredrick Institute of Plant Protection and Toxicology, Padappai, Tamil Nadu, India. Rats were maintained under hygienic conditions and were provided a diet of standard rat pellets [Hindustan Lever Ltd., Bombay, India] and water *ad libitum*. Animals (n = 12) were divided into an experimental and control group. The control group was given sunflower oil only. The experimental rats were orally administered MeAN dissolved in Sunflower Oil [100 mg/kg body weight/day] daily over a 14 day period according to Farooqui *et al.* (1990). At the end of the test period, the blood was collected by cutting the jugular vein and lungs were removed. Serum was separated by centrifugation.

To assay antioxidant enzymes, 100 mg of lung tissue was homogenized in 1.15% KCl. Homogenates were centrifuged at 3000 rpm for 30 min at 4°C. The supernatant was used to measure glutathione (GSH) (Moron *et al.* 1979), catalase (CAT) (Caliborne 1985), superoxide dismutase (SOD) (Misra and Fridovich 1976), glutathione reductase (GSSR) (Dulber and Anderson 1981) glutathione s-transferase (GST) (Habig *et al.* 1974), glutathione peroxidase (GPX) (Rotruk *et al.* 1973), total sulfhydryl (T-SH) (Sedlack and Lindsay 1968), glucose-6-phosphatedehydrogenase (G6PD) (Ralinsky and Rernstein, 1963), serum and lung tissue lipid peroxides (Ohkawa *et al.* 1978) and protein (Lowry *et al.* 1951).

A student's 't' test was used to examine differences in enzyme levels between experimental and control groups. P value ≤ 0.05 was considered significant.

RESULTS AND DISCUSSION

The lung homogenate and serum from rats treated with MeAN showed increased levels of thio barbituric acid (TBA) reactive substances ($p<0.001$) and glutathione-s-transferase ($p<0.001$, Table 1). The levels of glutathione, glutathione peroxidase ($p<0.01$), and total sulfhydryl ($p<0.001$) were reduced (Table 1). The activities of glucose 6-phosphate dehydrogenase and catalase were reduced significantly whereas the superoxide dismutase activity was significantly increased and glutathione redactase remained unchanged following exposure to MeAN.

Glutathione (GSH), a pulmonary cytosolic low molecular weight tripeptide, plays an important role in the MeAN detoxification mechanism. MeAN undergoes conjugation with reduced glutathione in a reaction that could be catalyzed by glutathione-s-transferase (GST)

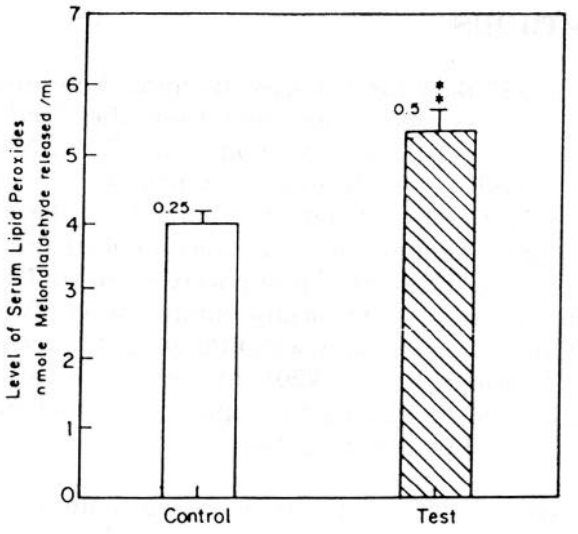

Figure 1. Serum Lipid peroxide in Control and Methacrylonitrile treated Rats.
Values are expressed as mean ± SD for 6 rats in each group. ** p<0.01.

and/or proceed nonenzymatically (Silver *et al.* 1982; Ghanayem *et al.* 1994). An earlier study also reported the depletion of non-protein sulfhydryls (reduced glutathione) by MeAN *in vivo* (Day *et al.* 1988) and *in vitro* (Meister 1988). Glutathione has the capacity to scavenge oxidizing species directly by virtue of its strong reducing thiol group. It has been postulated to be an important component of the defense against oxidative injury to the lung. The depletion of GSH could have resulted in the impaired clearance of $O_2^{\cdot}$, OH•, and H_2O_2 which could have resulted in increased levels of lipid peroxidation products in the lungs of animals treated with MeAN (Comporti M 1987). An increase in serum lipid peroxidation products (Fig.1) observed in this study is thought to be the consequence of increased production of tissue lipid peroxidation products (Yagi 1987) and their liberation into the circulation.

Superoxide dismustase (SOD) and catalase (CAT) scavenge $O_2^{\cdot}$ and H_2O_2, which initiates lipid peroxidation (Halliwell and Gutteridege 1989). The increased SOD and reduced CAT in MeAN-treated rat lungs would have led to the accumulation of H_2O_2. The lung tissue has the lowest catalase activity and therefore is one of the highly susceptible organs for peroxidative damage. The reduction in GPx activity, the enzyme which reduces lipid peroxidation products and H_2O_2 utilizing GSH as the co-substrate (McCay *et al.* 1976), also leads to an accumulation of H_2O_2 and lipid peroxidation products. Under conditions of GSH depletion by

MeAN, it may be expected that (GPx) cannot handle the excessively generated H_2O_2 and lipid peroxidation products.

Table 1. Levels of Lipid peroxides, glutathione-s-transferase, glutathione, glutathione peroxidase, total sulfhydryl, protein, catalase, superoxide dismutase, glucose-6-phosphate dehydrogenase and glutathione reductase in the lung of control and MeAN-treated rats (100mg/kg/body wt) for 14 days.

Antioxidant Enzyme Acitivity	Control	MeAN treated
Lipid peroxides (nmol MDA released/mg protien)	43.7 ± 10.5	79.5 ± 12.7***
Glutathione-s-transferase (nmole [1-culora-2,4- dinitro benzene] conjugated/ min/mg protein)	0.38 ± 0.03	0.75 ± 0.08***
Glutathione (nmole/mg protein)	1.78 ± 0.06	1.38 ± 0.05**
Glutathione peroxidase (µg GSH consumed/min/mg protein)	0.16 ± 0.05	0.08 ± 0.01**
Total sulfhydryl (nmoles of glutathione/mg protein)	76.9 ± 8.1	59.1 ± 8.7**
Protein (mg/g tissue)	166 ± 29	198 ± 10^{NS}
Catalase (µmol/H_2O_2 consumed/min/mg protein)	137 ± 17.8	73.6 ± 2.9***
Superoxide dismutase (Units/mg protein)	8.4 ± 0.8	11.6 ± 1.2***
Glucose-6-phosphate dehydrogenase (Units/mg protein)	1.15 ± 0.16	0.67 ± 0.32***
Glutathione reductase (µ moles NADPH utilized (min/mg protein)	39.2 ± 2.8	34.3 ± 4.2^{NS}

Values are expressed as mean ± SD for 6 rats in each group. ***$p<0.001$, **$p<0.01$ and NS-Non significant.

Glutathione-s-transferase (GST) plays an important role in the detoxification of xenobiotics utilizing as a substrate (Kosower 1976). MeAN is metabolized by conjugation with GSH in a reaction catalyzed by GST (Silver *et al.* 1982; Ghanayem *et al.* 1994). The observed increase in GST activity and a depletion of GSH in this study suggests that a major portion of MeAN is eliminated by conjugation with GSH and protein thiol groups, catalyzed by GST.

Glucose-6-phosphate dehydrogenase (G6PD) is an important antioxidant enzyme that generates Nicotinamide adenine dinucleotide phosphate (reduced) (NADPH) for the regeneration of GSH from GSSG (Jacob and Jaudi, 1966). Adequate supplies of NADPH are required for the activities of GPx and GR. The decrease in G6PD activity could have resulted in a reduction in NADPH, leading to the observed reduction in GPx. However, the GR activity remained unaltered on MeAN administration. This suggests that the insufficient supply of NADPH by the Hexose monophosphate (HMP) shunt pathway due to a reduction of GGPD activity leads to an increase in GSSG concentration since GR cannot keep up with the rate of GSH oxidation. This is followed by reduced levels of cellular GSH pool in MeAN treatment.

The depletion of GSH and impairment of cellular antioxidant enzymes were observed in the present study. We propose that these contribute to the accumulation of lipid peroxidation products and increase the susceptibility of the MeAN-treated rat lung to peroxidative damage.

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