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Does Methyl Isocyanate Interaction with Normal Hemoglobin Alter Its Structure and Function?

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The predominant biological effect of methyl isocyanate (MIC) intoxication in mammals is severe tissue hypoxia leading to acute lactic acidosis (Jeevarathinam et al 1988; Jeevaratnam et al 1990). In rabbits administered MIC subcutaneously (s.c.) the hypoxia was shown to be of the stagnant type resulting from hypovolemic hypotension (Jeevarathinam et al 1988). Lee (1976) showed in his in vitro studies that MIC has anti-sickling activity by virtue of its reaction with the amino-terminal of hemoglobin (Hb) forming the methyl carbamylated Hb-S (sickle) which showed an increased oxygen affinity but the effect of MIC on normal Hb was not investigated. The in vitro experiments by Lee (1976) prompted researchers to hypothesize that MIC carbamylation significantly increased the Hb-O affinity which inhibited peripheral oxygen unloading resulting in tissue hypoxia. Recently, the occurrence of carbamylation of Hb by MIC in vivo was demonstrated unequivocally (Ramachandran et al 1988; Bhattacharya et al 1988). Furthermore, the characteristic observation, dark red colored (cherry red) blood in animals exposed to MIC remained unexplained. This prompted us to investigate whether MIC exposure caused an alteration in structure and/or function of normal Hb leading to tissue hypoxia and the change in the color of the blood.

MATERIALS AND METHODS

Methyl isocyanate (99% purity) was synthesised in the Chemistry Division of Defence R&D Establishment and characterised by proton NMR, IR and mass spectrometry. MIC was dissolved in olive oil for injection purposes. For the studies on the effects of MIC on Hb function, in vivo, rabbits were anesthetised with urethane (2 g/kg body weight). Femoral artery and vein were cannulated for repeated blood sampling in an heparinized air tight syringe for blood gas analysis in ABL3 acid base laboratory equipment. Samplings were done prior to and at 30, 60, 90, 120 and 180 min after either olive oil (control) or MIC administration s.c. at two doses, 0.5 and 1.0 LD50, namely, 63 and 126 mg/kg respectively (Jeevarathinam et al 1988).

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For in vitro studies, chilled aqueous solutions of MIC were prepared and used within 2 min. The stability of MIC in aqueous solution (chilled) appeared to be stable for 2 min as analysed by gas chromatography while only 70, 50 and 23 percent of MIC in aqueous solutions at 25°C could be detected at 2, 15 and 30 min respectively (Meshram and Rao 1988). Rabbit Hb was prepared by hemolyzing washed erythrocytes with 0.01 M phosphate buffer, pH 7.4 and no further purification was done (Sugita et al 1971). For in vitro studies, Hb solutions (2.25×10^{-5} M) in 0.1 M phosphate buffer were taken in tubes to which MIC in aqueous solution was added to have MIC concentrations at 125 uM, 1.25 mM and 2.5 mM. Tubes were incubated at 25°C for 30 min, 4 hr, 12 hr and 24 hr. For in vivo studies, Hb samples were prepared from blood drawn from rabbits administered 1.0 LD50 MIC s.c. at 1 hr, 4 hr and 24 hr.

Electrophoresis of the samples was done on cellulose acetate strips using Tris-EDTA- borate buffer, pH 8.6. After the run for 2 hours with a current of 0.4 mA/cm, the strips were dried, stained using either Ponceau S in TCA or O-dianisidine and hydrogen peroxide (Marengo-Rowe 1965).

Absorption spectra of oxy-Hb of all in vitro samples were measured with a Shimadzu UV-Vis spectrophotometer while circular dichroism (CD) spectral measurements were performed at 25°C on a Jasco ORD/CD spectropolarimeter. All measurements were performed in 0.1 M phosphate buffer, pH 7.4 (Sugita et al 1971). The Hb samples of both in vitro and in vivo experiments were also scanned for the presence of methemoglobin (metHb) and cyanmethemoglobin (cyanmetHb) in Pye Unicam SP-8-500 UV-Vis Spectrophotometer.

RESULTS AND DISCUSSION

Methyl isocyanate was shown to possess anti-sickling activity by virtue of its reaction with amino-terminal of sickle Hb forming methyl carbamylated Hb-S, which showed an increased oxygen affinity (Lee 1976). In contrast, we found an enhanced oxygen release (i.e., decrease in oxygen affinity) in rabbits administered MIC s.c. as the arterio-venous difference of oxygen content getting increased with the progression of time and also the venous pO_2 is decreased (Fig 1). This can be attributed to the observed acidosis and increased in blood 2,3-diphosphoglycerate levels (Jeevarathinam et al 1988). Maginniss et al (1987) also observed a reduction of blood oxygen affinity in guinea pigs exposed to MIC vapors. These observations are likely to mislead, as the quantum of carbamylation occurred in vivo was too low (detectable only by GC analysis) to bring about any depictable functional derangement even if MIC causes alteration in structure and/or function of Hb. Hence, we have exposed Hb samples to MIC in vitro and subjected to electrophoresis to ensure the presence of sizeable amount of methyl carbamylated Hb as depicted by altered electrophoretic

mobility (data not given), in conformity with the observation made by Troup et al (1987). The absorption and CD spectral analysis of all the Hb samples exposed to MIC at different concentrations for varied time intervals showed that MIC has not caused any appreciable change in the quarternary structure or conformation of oxy-Hb molecule (Fig 2,3).

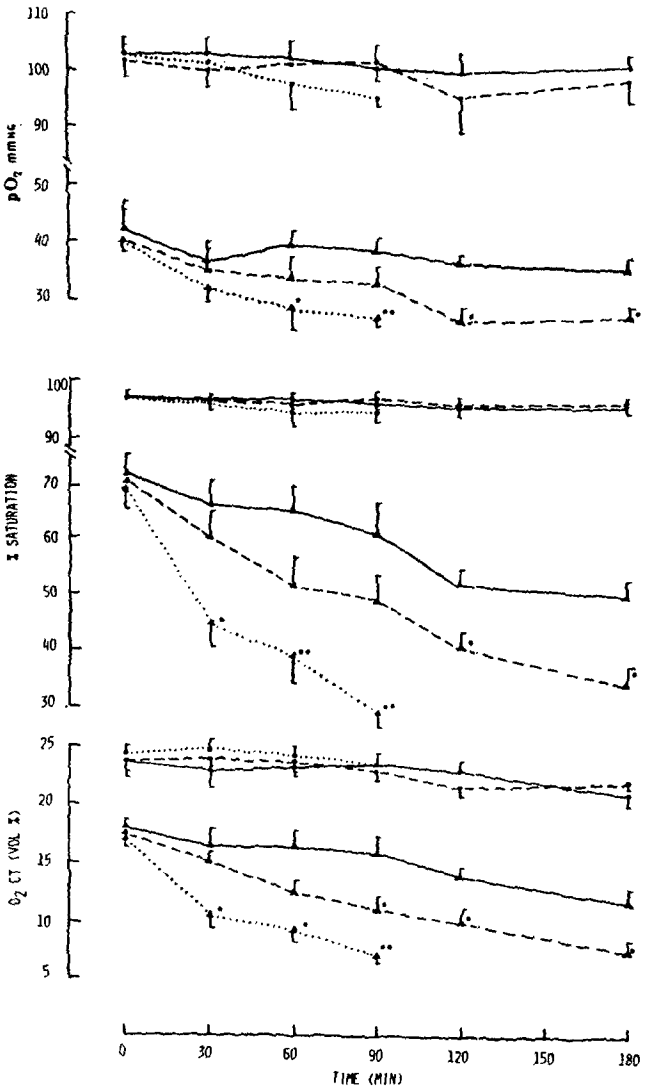

Figure 1. Effects of MIC on both arterial (•) and venous (▲) pO_2 , percent saturation and total oxygen content. Mean (±SE), n=6-8; control(—), 0.5 LD50 MIC (---) and 1.0 LD50 MIC (....) * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. [Reproduced with permission from K. Jeevarathinam et al (1988) Toxicology, 51:223-240]

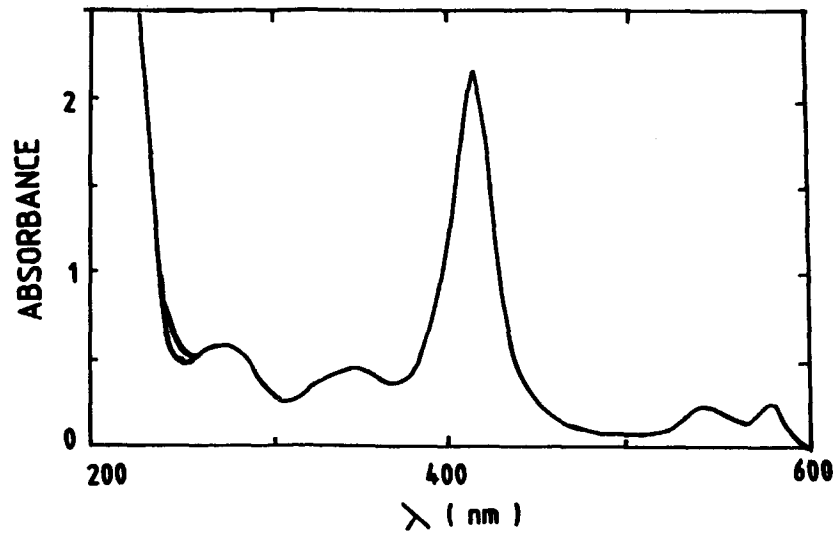

Figure 2. Absorbtion spectra of Oxy-hemoglobin, control and MIC treated (1.25 mM, 2.5 mM).

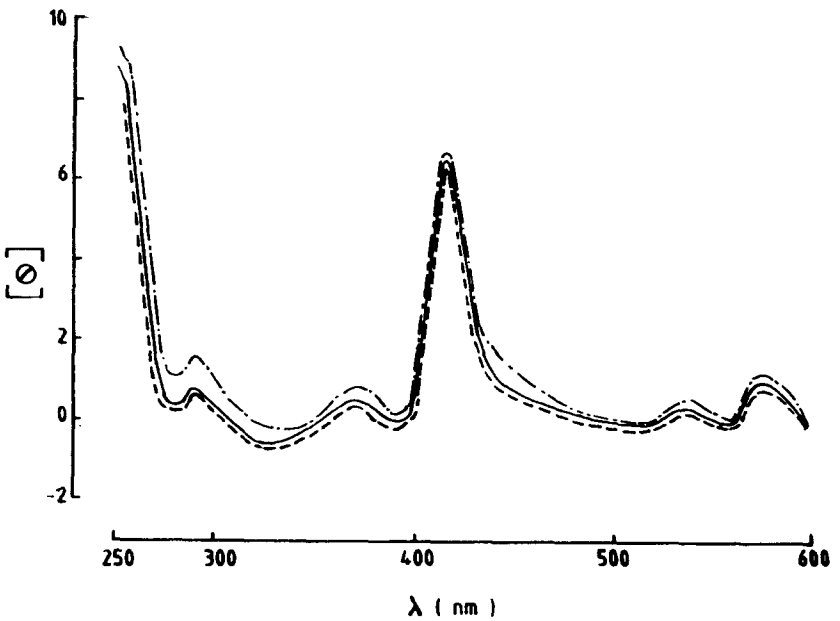

Figure 3. Circular dichroism spectra of Oxy-hemoglobin control (—), 1.25 mM MIC (---) and 2.5 mM MIC (-·-).

A characteristic observation made by us and others is that the blood samples from animals exposed to MIC vapors or administered parenterally appeared dark red colored (cherry red). This observation led us to ask whether MIC interaction with Hb may lead to formation of met-Hb and cyanmet-Hb (Lepkowski 1985). Several investigators, however, ruled out the

involvement of cyanide in the toxicity of MIC (Nemery et al 1985; Bucher et al 1987; Vijayaraghavan and Kaushik 1987). Still, the color change remained unexplained. This led us to screen all the Hb samples (both in vitro and in vivo) for the presence of met-Hb and cyanmet-Hb. Figure 4 (representative data) shows that MIC interaction with Hb does not lead to formation of either met-Hb or cyanmet-Hb. The dark red color observed in blood samples from MIC treated animals was not due to any abnormality ensuing from MIC interaction with Hb but probably due to the hemoconcentration (Jeevaratnam et al 1990) and/or increased deoxy Hb content (Fig 1).

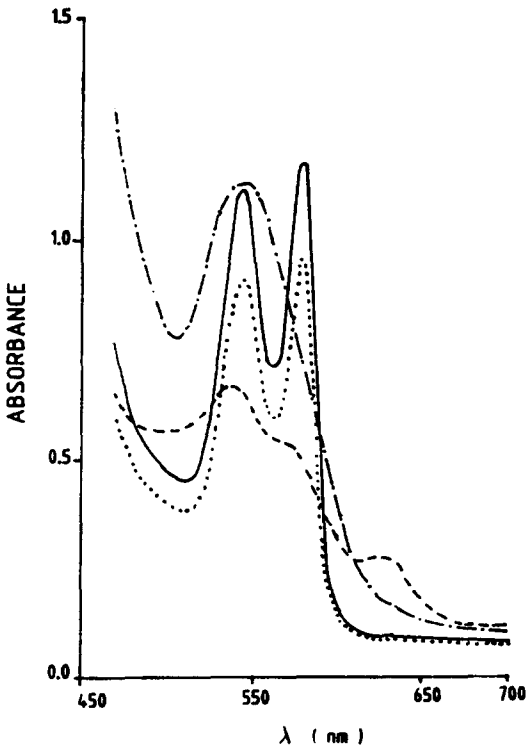

Figure 4. Absorption spectra of Oxy-hemoglobin, control (—), MIC treated (...), methemoglobin (---) and cyanmethemoglobin (-·-).

In conclusion, MIC does not cause any appreciable alteration in structure and function of normal Hb except for a qualitative change in the electrophoretic mobility of Hb on cellulose acetate strips. Also MIC interaction with Hb molecules does not play a role in the production of hypoxia in mammals, in vivo.

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