Biological Effects of Short-Term, High-Concentration Exposure to Methyl Isocyanate. IV. Influence on the Oxygen-Binding Properties of Guinea Pig Blood

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Whole blood oxygen equilibrium curves (O₂ ECs), blood buffer lines, and several hematologic properties were determined for adult guinea pigs exposed to 700 ppm methyl isocyanate (MIC) for 15 min. MIC inhalation effected a significant reduction of blood O₂ affinity; the half-saturation pressure (P₅₀) at 38°C increased from the control (untreated) level of 22.8 ± 0.1 mm Hg to values ranging from 25.5 to 43.7 mm Hg for experimental animals. MIC exposure had no apparent influence on O₂ EC shape or CO₂ Bohr effect. Erythrocyte volume, [methHb], O₂ binding capacity, and combined red cell organic phosphate concentration (DPG + ATP) were not affected by MIC treatment. However, experimental animals experienced a severe metabolic acid-base disturbance; blood lactate concentration ranged from 8.6 to 24.0 mmole/L. Results indicate that lactic acidosis was solely responsible for increased blood P₅₀ of MIC-treated animals. No direct effects of MIC on hemoglobin function were observed. Reduced Hb-O₂ affinity, in conjunction with severe hypoxemia, compromised the guinea pigs' capacity for pulmonary O₂ loading; at Pao₂ of 30 mm Hg, Hb-O₂ saturation (S) decreased from 66% S for controls to 42% S for MIC-treated animals.

Introduction

Carbamylation of amino terminal residues of hemoglobin (Hb) by cyanate compounds alters the physical and functional properties of the protein tetramer (1). Cyanate and isocyanate have been investigated extensively as potential anti-aggregation agents for treatment of sickle cell disease (2,3). The effect of cyanate carbamylation on Hb-O₂ binding has also provided a valuable research tool for testing the adaptive significance of increased blood oxygen affinity for high altitude exposure (4,5). These reported effects of cyanates on Hb-O₂ transport prompted speculation that methyl isocyanate (MIC) impaired tissue oxygen delivery among victims of the Bhopal tragedy. This hypothesis suggested that MIC carbamylation significantly increased Hb-O₂ affinity, which inhibited peripheral O₂ unloading and resulted in tissue hypoxia.

This investigation reports the effects of MIC inhalation at a high and lethal concentration on the blood oxygen transport properties of spontaneously breathing guinea pigs. Results showed a notable reduction of Hb-O₂ affinity caused by hypoxia-induced lactic acidosis. A direct effect of isocyanate on hemoglobin function (i.e., carbamylation) was not detected.

Materials and Methods

Animals, Treatment, and Blood Collection

Adult female guinea pigs (Hartley strain) weighing 424 to 568 g were exposed to a mean methyl isocyanate concentration of 698 ppm (range 618–804 ppm) for 15 min. A detailed description of methods for MIC treatment is presented elsewhere (6). Immediately following exposure, animals were lightly anesthetized with Halothane and blood drawn from the retro-orbital sinus into heparinized Vacutainers (Becton-Dickinson, Rutherford, NJ). Control guinea pigs were exposed to air alone and bled in an identical manner. Blood samples were immediately packed in ice and transported to Brown University by air. Experimental measurements commenced approximately 5 hr after blood collection.

Oxygen Equilibrium Curves (O₂ ECs)

Multiple-point isocapnic O₂ ECs were generated for whole blood of control and experimental guinea pigs at
38°C by using microtechniques previously described (7). Briefly, a small aliquot of blood (0.5 to 1.0 μL) was gently spread between gas-permeable Teflon membranes and the blood-membrane trilayer secured by O-ring to an opaque carrier-disk with 7 mm center hole. The blood film was then mounted horizontally in a single compartment sample chamber (1 mL internal volume) and equilibrated with a humidified CO₂/N₂ gas mixture. Following desaturation, the blood sample was equilibrated with 24 to 34 (X = 28) isocapnic gas mixtures of increasing O₂ tension. For each static point, blood film P₀₂ was determined by measuring the O₂ tension of the surrounding gas phase by electrode oximetry. Simultaneously, Hb-O₂ saturation (S) was determined by dual wavelength spectrophotometry (542, 560 nm), light being transmitted to and from the blood film by optical fiber bundles. When O₂ tension in the cuvette produced a saturation greater than 95% S, the blood film was exposed to CO₂/O₂ (P₀₂ > 600 mm Hg) to obtain a 100% S signal. Complete O₂ ECs were generated in approximately 20 min, and data were transmitted directly to an IBM PC programmed for data acquisition and analysis. A fresh blood film was prepared for each O₂ EC to minimize the potential effects of erythrocyte metabolism on blood O₂ affinity.

Three isocapnic O₂ ECs were measured for each blood sample at 2, 5, and 8% CO₂. Blood film pH was estimated for each equilibrium curve from two-point Astrup blood buffer lines (8) determined with a microtonometer (AMT1, Radiometer, Copenhagen), thermostatted glass electrode, and pH meter (pH M, Radiometer). P₀₂ values were read for each O₂ EC at 5% saturation increments between 5 and 95% S. CO₂ Bohr coefficients (Δ log P₀₂/ΔpH) were then determined by least-square regression (5–95% S), and a standard O₂ EC was calculated for each individual at the appropriate blood pH or PCO₂.

Hematologic Properties

Hematocrit was determined by centrifugation at 13,000g for 6 min in heparinized capillaries. Hemoglobin concentration [Hb] was measured as cyanmethemoglobin at 540 nm (Sigma Chem. Co., St. Louis, MO; Tech. Bull. 525) and [metHb] by the method of van Assendelft (9) at 630 nm. O₂ capacity (Lex-O₂-Con, Waltham, MA) was determined for air-equilibrated samples of whole blood (P₀₂ = 150 mm Hg) and corrected for dissolved O₂ (10). DPG, ATP, and lactate concentrations were determined by enzymatic assay (Sigma Tech. Bull. 35-UV, 366-UV, and 826-UV, respectively).

Results and Discussion

Blood Oxygen-Binding Properties

Figure 1 illustrates O₂ ECs for blood of control and MIC-treated guinea pigs at 38°C. The P₀₂ at half-saturation (P₅₀) for control animals at pH 7.40 was 22.8 ± 0.1 mm Hg (X ± 1 SEM, N = 5). This O₂ affinity coefficient was somewhat lower than P₅₀ values previously reported for guinea pigs (11). Differences may be related to animal age, methods of anesthesia and/or experimental techniques for generating equilibrium data. O₂ EC values for MIC-treated animals at a common PCO₂ of 40 mm Hg were significantly right-shifted and exhibited substantial individual variability (Fig. 1). The P₅₀ for the five experimental animals ranged from 28.5 to 43.7 mm Hg.

Figure 2 illustrates the effects of methyl isocyanate exposure on the shape of the O₂ equilibrium curve. For this analysis, the individual O₂ ECs for experimental animals were scaled to the control P₅₀ (22.8 mm Hg). (δ) define the P₀₂ range for the five scaled data sets between 5 and 95% S; (—) is mean control O₂ EC shown in Fig. 1. Results indicate no apparent effect of MIC inhalation on O₂ EC shape.
The CO₂ Bohr effect at half-saturation (Δ log P₅₀/ΔpH) was not different for control (−0.62 ± 0.03) and MIC-treated animals (−0.60 ± 0.05). CO₂ Bohr slopes were also saturation-independent between 10 and 90% S for both animal groups.

Results of these oxygen-binding studies revealed that MIC inhalation significantly increased P₅₀ but had no influence on O₂ EC shape or the effect of carbon dioxide on blood O₂ affinity. Several hematologic properties relevant to blood oxygen transport were evaluated to determine the factor(s) responsible for the decreased Hb-O₂ affinity.

Hematologic Properties

MIC inhalation for 15 min at a concentration of 700 ppm produced significant increases in hematocrit ratio and [Hb] (Table 1). The mean corpuscular hemoglobin concentration (MCHC), however, remained unchanged (Table 1). These findings suggest that MIC treatment had no effect on erythrocyte volume. Reduced Hb-O₂ affinity of experimental animals, therefore, cannot be attributed to the potential consequences of cell volume change, i.e., effects of volume-induced changes in [Hb] (12) and intracellular pH (13). Furthermore, methyl isocyanate exposure did not promote Hb oxidation; [metHb] was approximately 1% of total [Hb] for both animal groups (Table 1).

MIC treatment had no effect on oxygen binding capacity of guinea pig blood (Table 1); the slightly higher capacity value reported for experimental animals reflects their increased [Hb]. The calculated oxygen to hemoglobin ratio (mL O₂/g Hb) for air-equilibrated blood samples was approximately 1.3 for both control and MIC-treated animals.

The organic phosphates DPG and ATP, important allosteric modifiers of Hb function, exhibited small but significant differences between animal groups (Table 1). MIC-treated animals had decreased [DPG] and increased [ATP]. The net effect was a minimal change in combined erythrocyte organic phosphate concentration. These observed changes in RBC organic phosphates are consistent with severe acidosis (1).

MIC-treated guinea pigs experienced a metabolic acid-base disturbance. Blood lactate concentrations among these spontaneously breathing animals ranged from 8.6 to 24.0 mmole/L (Table 1). Blood gas and acid-base measurements also revealed a metabolic acidosis for pump-ventilated guinea pigs following 15 min exposure to 675 ppm MIC (14). [Lactate] for control animals was also elevated (2.6–7.4 mmole/L); these latter findings may reflect a metabolic acid-base disturbance resulting from halothane-induced ventilatory depression.

Effect of Metabolic Acidosis on Hb-O₂ Affinity

Increased blood [lactate] resulting from methyl isocyanate inhalation was apparently the sole cause for the observed reduction of Hb-O₂ affinity among the experimental animals. The O₂ EC for MIC-treated guinea pigs are reported at a standard mammalian arterial P CO₂ of 40 mm Hg (Fig. 1). The corresponding blood pH values ranged from 7.19 to 6.79, reflecting the severe metabolic acidosis. Furthermore, there was a direct relationship between blood [lactate] and P₅₀ for MIC-treated guinea pigs, i.e., animals with the highest [lactate] exhibited the highest O₂ affinity coefficient.

The effect of metabolic acidosis on P₅₀ was evaluated by calculating the O₂ affinity coefficients for experimental animals at blood pH 7.40 using the measured CO₂ Bohr slopes. At pH 7.40, the half-saturation P O₂ for MIC-treated animals (20.7 ± 0.7 mm Hg) approximated the control P₅₀ (22.8 ± 0.1 mm Hg). In a more definitive study, blood from three experimental guinea pigs was titrated to the control base excess with NaHCO₃. The measured P₅₀ for titrated blood from experimental animals (22.9 ± 1.3 mm Hg at pH 7.40) was virtually identical to the control value. These findings strongly suggest that the reduced Hb-O₂ affinity in MIC-treated animals resulted from the lactic acidosis.

Functional Consequences of MIC Treatment on Blood O₂ Delivery

MIC inhalation (675 ppm) caused rapid and severe lung injury (15), resulting in significant intrapulmonary shunts and ventilation-perfusion mismatch (14). The functional consequences of this pulmonary damage was hypoxemia; arterial P O₂ ranged from 35 to 40 mm Hg for pump-ventilated animals following 15 min MIC exposure (14). For spontaneously breathing guinea pigs, a lower PaO₂ would be predicted. The present investigation also revealed a metabolic acid-base disturbance; blood [lactate] in the MIC-treated animals was significantly elevated (Table 1). These latter findings are indicative of tissue hypoxia. Systemic O₂ delivery for the MIC-treated guinea pig was apparently inadequate to sustain the animal's aerobic energy requirements, necessitating the added contribution of anaerobic glycolysis.

The acid-induced reduction of Hb-O₂ affinity, in con-
junction with severe hypoxemia, further jeopardized
the guinea pigs' capacity for blood oxygen transport. To
substantiate this conclusion, Hb-O₂ saturation was cal-
culated for control and experimental animals at an
assumed PaO₂ of 30 mm Hg. At pHₐ 7.40, control gui-
nea pig blood would be 66% saturated with oxygen
at PaO₂ = 30 mm Hg. For MIC-treated animals (P₈CO₂ = 40 mm Hg), the right-shifted equilibrium curve
would reduce arterial saturation to 42%, values ranging
from 31 to 53% S for the five individuals. This analysis
assumes only a metabolic acid–base disturbance for ex-
perimental animals. Inclusion of the respiratory acidosi
reported for MIC-exposed guinea pigs (14) would fur-
ther right-shift the O₂ EC, reduce arterial saturation to
a lower level, and hence further compromise pul-
monary oxygen loading.

This investigation provided no evidence for a direct
effect of methyl isocyanate on hemoglobin function. Al-
though MIC is highly reactive with Hb when blood is
exposed in vitro (3, 16), the reported effect of carba-
mation on Hb–O₂ affinity was not detected for inha-
lation-treated guinea pigs. One interpretation of these
findings suggests that the rapid and devastating effects
of high MIC concentrations on pulmonary structure
(15), blood-gas exchange properties (14), and possible
reflex inhibition of breathing (17) minimized the effec-
tive contact of the gas with functional alveoli.

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