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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 (O_2 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 O_2 affinity; the half-saturation pressure (P_{50}) at 38°C increased from the control (untreated) level of 22.8 ± 0.1 mm Hg to values ranging from 28.5 to 43.7 mm Hg for experimental animals. MIC exposure had no apparent influence on O_2 EC shape or CO_2 Bohr effect. Erythrocyte volume, [metHb], O_2 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 P_{50} of MIC-treated animals. No direct effects of MIC on hemoglobin function were observed. Reduced Hb-O_2 affinity, in conjunction with severe hypoxemia, compromised the guinea pigs' capacity for pulmonary O_2 loading; at Pa_{O_2} of 30 mm Hg, Hb-O_2 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-O_2 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-O_2 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-O_2 affinity, which inhibited peripheral O_2 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-O_2 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 (O_2 ECs)

Multiple-point isocapnic O_2 ECs were generated for whole blood of control and experimental guinea pigs at

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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 CO_2/N_2 gas mixture. Following desaturation, the blood sample was equilibrated with 24 to 34 ($\bar{X}$ = 28) isocapnic gas mixtures of increasing O_2 tension. For each static point, blood film P_{O_2} was determined by measuring the O_2 tension of the surrounding gas phase by electrode oximetry. Simultaneously, Hb-O_2 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 O_2 tension in the cuvette produced a saturation greater than 95% S, the blood film was exposed to CO_2/O_2 ($P_{O_2} > 600$ mm Hg) to obtain a 100% S signal. Complete O_2 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 O_2 EC to minimize the potential effects of erythrocyte metabolism on blood O_2 affinity.

Three isocapnic O_2 ECs were measured for each blood sample at 2, 5, and 8% CO_2. 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 (pHM 84, Radiometer). P_{O_2} values were read for each O_2 EC at 5% saturation increments between 5 and 95% S. CO_2 Bohr coefficients ($\Delta \log P_{O_2}/\Delta pH$) were then determined by least-square regression (5–95% S), and a standard O_2 EC was calculated for each individual at the appropriate blood pH or P_{CO_2}.

Hematologic Properties

Hematocrit was determined by centrifugation at 13,000*g* for 6 min in heparinized capillaries. Hemoglobin concentration [Hb] was measured as cyanomethemoglobin at 540 nm (Sigma Chem. Co., St. Louis, MO; Tech. Bull. 525) and [metHb] by the method of van Assendelft (*9*) at 630 nm. O_2 capacity (Lex-O_2-Con, Waltham, MA) was determined for air-equilibrated samples of whole blood ($P_{O_2} \simeq 150$ mm Hg) and corrected for dissolved O_2 (*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 O_2 ECs for blood of control and MIC-treated guinea pigs at 38°C. The P_{O_2} at half-saturation (P_{50}) for control animals at pH 7.40 was 22.8 ± 0.1 mm Hg ($\bar{X}$ ± 1 SEM, N = 5). This O_2 affinity coefficient was somewhat lower than P_{50} 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. O_2 EC values for MIC-treated animals at a common P_{CO_2} of 40 mm Hg were significantly right-shifted and exhibited substantial individual variability (Fig. 1). The P_{50} for the five experimental animals ranged from 28.5 to 43.7 mm Hg.

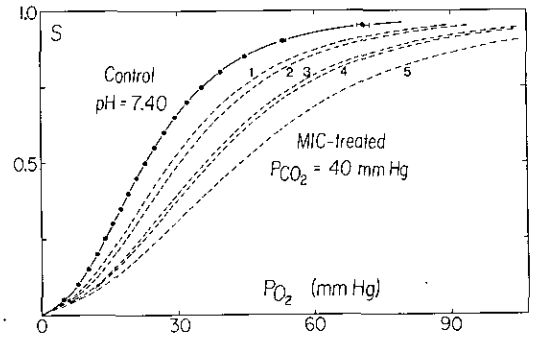

FIGURE 1. O_2 equilibrium curves (O_2 ECs) for guinea pig whole blood at 38°C measured by thin-film techniques (*7*). Control curve is mean O_2 EC for five individuals at pH 7.40; (⊢⊣) ± 1 SEM; (---) O_2 ECs for five MIC-treated guinea pigs at blood P_{CO_2} = 40 mm Hg. Corresponding blood pH values for experimental data were: (*1*) 7.194; (*2*) 7.184; (*3*) 6.966; (*4*) 6.935; (*5*) 6.790.

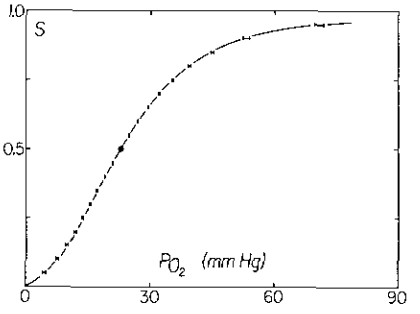

FIGURE 2. Graphical method for comparing O_2 EC shape for control and MIC-treated guinea pigs. For this analysis, equilibrium data for experimental animals were scaled to the control P_{50} (22.8 mm Hg). (‖) define the P_{O_2} range for the five scaled data sets between 5 and 95% S; (⊢⊣) is mean control O_2 EC shown in Fig. 1. Results indicate no apparent affect of MIC inhalation on O_2 EC shape.

Figure 2 illustrates the effects of methyl isocyanate exposure on the shape of the O_2 equilibrium curve. For this analysis, the individual O_2 ECs for experimental animals were scaled to the control P_{50} (22.8 mm Hg). The vertical bars, plotted at 5% saturation increments between 5 and 95% S, encompass the P_{O_2} ranges for the five MIC-treated data sets. The solid curve is the mean O_2 EC for control animals illustrated in Figure 1. Results indicate minimal P_{O_2} variability among the five scaled experimental O_2 ECs. Furthermore, the normalized data described an O_2 EC shape almost identical to the control curve. MIC inhalation, at a high and lethal concentration, had no apparent effect on equilibrium curve shape.

The CO_2 Bohr effect at half-saturation ($\Delta \log P_{50}/\Delta pH$) was not different for control (-0.62 ± 0.03) and MIC-treated animals (-0.60 ± 0.05). CO_2 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 P_{50} but had no influence on O_2 EC shape or the effect of carbon dioxide on blood O_2 affinity. Several hematologic properties relevant to blood oxygen transport were evaluated to determine the factor(s) responsible for the decreased Hb-O_2 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-O_2 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 O_2/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.

Table 1. Hematologic properties of guinea pig blood.

Property	Control[a]	MIC treated[a]	Probability[b]
Hematocrit, %	45.7 ± 0.6	50.7 ± 1.3	$p < 0.01$
[Hb], g/dL blood	15.4 ± 0.2	16.9 ± 0.3	$p < 0.005$
MCHC, g Hb/dL RBC	33.6 ± 0.2	33.3 ± 0.4	NS
[metHb], % total Hb	1.1 ± 0.2	1.2 ± 0.2	NS
O_2 capacity, mL O_2/dL blood[c]	19.6 ± 0.4	21.0 ± 0.5	NS
[DPG], mmole/L RBC	6.82 ± .05	6.44 ± .14	$p < 0.05$
[ATP], mmole/L RBC	0.51 ± .04	0.71 ± .03	$p < 0.005$
[lactate], mmole/L blood	5.1 ± 0.8	15.4 ± 2.9	$p < 0.01$

[a] Mean ± SEM ($n = 5$).
[b] t-test statistic for unpaired data.
[c] Blood O_2 capacity determined for three control and three MIC-treated animals.

Effect of Metabolic Acidosis on Hb-O_2 Affinity

Increased blood [lactate] resulting from methyl isocyanate inhalation was apparently the sole cause for the observed reduction of Hb-O_2 affinity among the experimental animals. The O_2 EC for MIC-treated guinea pigs are reported at a standard mammalian arterial P_{CO_2} 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 P_{50} for MIC-treated guinea pigs, i.e., animals with the highest [lactate] exhibited the highest O_2 affinity coefficient.

The effect of metabolic acidosis on P_{50} was evaluated by calculating the O_2 affinity coefficients for experimental animals at blood pH 7.40 using the measured CO_2 Bohr slopes. At pH 7.40, the half-saturation Po_2 for MIC-treated animals (20.7 ± 0.7 mm Hg) approximated the control P_{50} (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 $NaHCO_3$. The measured P_{50} 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-O_2 affinity in MIC-treated animals resulted from the lactic acidosis.

Functional Consequences of MIC Treatment on Blood O_2 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 Po_2 ranged from 35 to 40 mm Hg for pump-ventilated animals following 15 min MIC exposure (*14*). For spontaneously breathing guinea pigs, a lower Pa_{O_2} 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 O_2 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-O_2 affinity, in con-

junction with severe hypoxemia, further jeopardized the guinea pigs' capacity for blood oxygen transport. To substantiate this conclusion, Hb-O_2 saturation was calculated for control and experimental animals at an assumed Pa_{O_2} of 30 mm Hg. At pH_a 7.40, control guinea pig blood would be 66% saturated with oxygen at $Pa_{O_2} = 30$ mm Hg. For MIC-treated animals ($P_{CO_2} = 40$ 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 experimental animals. Inclusion of the respiratory acidosis reported for MIC-exposed guinea pigs (*14*) would further right-shift the O_2 EC, reduce arterial saturation to a lower level, and hence further compromise pulmonary oxygen loading.

This investigation provided no evidence for a direct effect of methyl isocyanate on hemoglobin function. Although MIC is highly reactive with Hb when blood is exposed *in vitro* (*3,16*), the reported effect of carbamylation on Hb-O_2 affinity was not detected for inhalation-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 effective contact of the gas with functional alveoli.

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