Effects of Almitrine Bismesylate in a Microswine Model of Hypoxemic Hypothermia

BRIAN J. GENTILE, MICHAEL J. DURKOT, BARBARA A. KRESTEL, INGRID V. SILLS, KIMBERLY A. TARTRARINI, DENISE A. ENGLISH, and AHMAD M. ALKHYYAT


We have developed an anesthetized microswine model of hypoxemic hypothermia and rewarming for testing prophylaxes and treatments. The respiratory stimulant almitrine bismesylate (ALM) was considered as a potential field expedient therapy for hypoxic hypothermia. Preliminary experiments demonstrated that five consecutive 100 μg·kg⁻¹ ALM intravenous (iv) doses given to normothermic microswine 3.4 min apart increased minute ventilation from an average of 3.4 L·min⁻¹ to 4.5 L·min⁻¹ (n = 2). However, when either a single iv ALM dose of 150 μg·kg⁻¹ (n = 1) or three consecutive 100 μg·kg⁻¹ iv doses given 15 min apart (n = 1) to hypoxic hypothermic microswine with a mean esophageal temperature (Te) = 28.8°C, and a mean arterial O₂ partial pressure (PaO₂) = 49 mmHg, the hypoxemia was potentiated (mean PaO₂ = 32 mmHg) and respiratory arrest ensued. Other experiments using continuous ALM iv infusion (1.0 μg·kg⁻¹·min⁻¹) in hypoxic hypothermic microswine (n = 6, Te = 30.6 ± 0.5, PaO₂ = 55.4 ± 12.9) did not demonstrate significant (p > 0.05) cardiopulmonary differences (ventilation, heart rate, blood pressure, blood gases) when compared to hypoxic hypothermic controls (n = 6, Te = 30.7 ± 0.5, PaO₂ = 53.3 ± 13.6). These results suggest that high dose iv bolus administration of ALM is not indicated as a potential field expedient therapy for hypoxic hypothermia, while further work is required to assess the potential efficacy of other continuous low dose iv infusion regimens.

Throughout history, hypothermia has been a major threat to military operations (6,14). This threat is magnified at altitude since ambient temperature decreases an average of 2.0°C for every 300 m of elevation (5); thus, soldiers deployed to mountainous terrain are subjected not only to hypoxia, but also the threat of hypothermia. At high altitude, the combination of hypoxia and hypothermia presents a serious hazard to individual and unit effectiveness.

Some of the physiologic effects of hypoxia include peripheral vasoconstriction, dehydration, and hemococoncentration (5), all of which are also induced by hypothermia (22). Thus, a higher incidence and greater severity of hypothermia-related injuries can be expected when altitude and cold are combined, and their effects are generally additive (12). Ultimately, death may occur consequent to hypoxemia, hypothermia and/or subsequent resuscitation.

When a severely hypothermic mountain casualty (core temperature < 30°C) is found, the potential for life-threatening cardiac arrhythmias is a primary concern because of the hyperirritability of the compromised myocaridum. Both hypoxia and hypothermia are noncardiogenic causes of ventricular arrhythmias, and ventricular fibrillation may be precipitated simply by the physical agitation of transport over rugged terrain to an evacuation helicopter (18,21). While supplemental oxygen can reduce this propensity for fibrillation (21), the logistical problems of transporting oxygen cylinders over mountainous terrain would render it, at best, an inconvenient field treatment, especially with multiple casualties. The advantages of a drug such as almitrine bismesylate (ALM, Vectarion®, Servier Laboratories, Orleans, France) are that it does not require sophisticated equipment for use, and can be rapidly administered to multiple casualties.

ALM is a rapidly-acting and persistent respiratory stimulant which increases pulmonary ventilation by specifically stimulating the carotid body (15,17), irrespective of the level of oxygenation (10). It has also been suggested that ALM improves the pulmonary ventilation/perfusion ratio by redistributing blood from poorly ventilated to better ventilated areas via finely regulated hypoxic pulmonary vasoconstriction (HPV) (16,20).

We hypothesized that ALM would increase arterial oxygenation in hypoxic hypothermic microswine by mechanisms related to peripheral chemoreceptor stimulation and/or HPV. If ALM has the potential to be a practical and effective field expedient treatment for hypoxic hypothermia allowing for safe patient transport, it would be a valuable countermeasure with immediate impact for treating altitude and cold illness and injury.

Methods

Animals

The Institutional Animal Care and Use Committee approved the experimental protocol describing this study.
HYPOXEMIC HYPOTHERMIA SWINE MODEL—GENTILE ET AL.

Fig. 1. Minute ventilation responses to hypoxic hypothermia and rewarming comparing effects of continuous ALM infusion (1.0 µg·kg⁻¹·min⁻¹) and PCB groups (n = 6). There were no differences between ALM and PCB groups. Values are means ± SD; Tₑₑₑ = mean esophageal temperature; * indicates significance from normothermic baseline; † indicates significance from both normothermic baseline and end rewarming (p < 0.05).

Prior to initiation. The animals were maintained under the surveillance of a veterinarian in a facility fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Microswine (Charles River Laboratories, Wilmington, MA) were used in this study for several reasons. The pathophysiology and biochemistry of swine have been well-documented (2,3,19). Unlike rats and rabbits, they have sufficient blood volume to allow the requisite consecutive samples. They were preferred over dogs because their physiology has been reportedly “closer” to humans (2,3), and are usually regarded as a non-pet species.

Eight barrows, 7-8 mo old, weighing 33.3 ± 2.2 kg were used. The animals were socially housed in fiberglass pens with slatted floors with ad lib access to water via an automatic watering system. Miniswine laboratory chow was given in two daily feedings.

Since splenic contraction is the primary cause of hemocoagulation in hypothermic pigs (11), the microswine were first splenectomized under isoflurane anesthesia for the experimental model preparation. The pig, unlike the human, has a large and contractile spleen capable of storing 20-25% of the total red cell volume (13). Thus, release of splenic-sequestered erythrocytes into the circulation during hypothermia and/or rewarming may result in effects that do not occur in humans because of their comparatively small (1-2%) splenic red cell reserve (1,4,8).

At 3-4 weeks following the splenectomy, 18 gauge Tygon (Norton Performance Plastics, Akron, OH) jugular venous and carotid arterial catheters were aseptically implanted from a neck incision under isoflurane anesthesia for blood sampling, and ALM or malic acid placebo (PCB) intravenous (iv) infusion. These catheters were flushed with sterile saline (0.9%), filled with heparin (1000 units·ml⁻¹), exteriorized at the back of the neck, then the incision was routinely closed. Catheter patency was maintained by flushing with sterile saline and filling with heparin every 2-3 d.

Postoperative analgesia for both surgical procedures was provided by butorphanol tartrate (0.5 mg·kg⁻¹, im) or nalbuphine HCl (1.0 mg·kg⁻¹, im) for at least the first two postoperative days. Naflaxin sodium (8 mg·kg⁻¹, iv) was administered intraoperatively, and once postoperatively as a prophylactic measure to offset the risk of infection from surgery. After catheterization and a 5- to 7-d recovery period, the experimental portion of the protocol was performed.

Physiological Measurements

Arterial blood pressure (Pₐ) was measured using a P23 ID transducer (Gould Electronics, Valley View, OH), and ventilation was measured using a 21072A pneumotach and 47304A flow transducer (Hewlett-Packard, Boise, ID). Tidal volume (V₁) and respiratory rate (RR) were integrated for determination of minute ventilation (Vₑₑₑ), corrected for body temperature and water vapor saturation (BTPS). Both Pₐ and Vₑₑₑ were recorded on a 2400S 4-channel physiological recorder (Gould Electronics, Valley View, OH). Esophageal temperature (Tₑₑₑ) was moni-

Fig. 2. a: Heart rate; and b: mean arterial pressure responses to hypoxic hypothermia and rewarming comparing the effects of continuous ALM infusion (1.0 µg·kg⁻¹·min⁻¹) to equivalent PCB infusion. There were no differences between ALM and PCB groups (n = 6). Values are means ± SD; Tₑₑₑ = mean esophageal temperature; * indicates significance from normothermic baseline (p < 0.05).
HYPOXEMIC HYPOTHERMIA SWINE MODEL—GENTILE ET AL.

TABLE I. ARTERIAL BLOOD GASES.

<table>
<thead>
<tr>
<th></th>
<th>Tem (°C)</th>
<th>$P_{aO_2}$ (mmHg)</th>
<th>$P_{aCO_2}$ (mmHg)</th>
<th>pH</th>
<th>$HCO_3$ (mmol·L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normothermic ALM</td>
<td>37.3 ± 0.3</td>
<td>57.9 ± 9.1</td>
<td>47.2 ± 2.2</td>
<td>7.44 ± 0.02</td>
<td>31.6 ± 2.1</td>
</tr>
<tr>
<td>Baseline PCB</td>
<td>37.1 ± 0.2</td>
<td>59.1 ± 5.1</td>
<td>47.6 ± 2.8</td>
<td>7.43 ± 0.01</td>
<td>31.6 ± 2.1</td>
</tr>
<tr>
<td>Hypothermic ALM</td>
<td>30.6 ± 0.5</td>
<td>55.4 ± 12.9</td>
<td>53.9 ± 6.2</td>
<td>7.39 ± 0.04</td>
<td>35.3 ± 2.4</td>
</tr>
<tr>
<td>Baseline PCB</td>
<td>30.7 ± 0.5</td>
<td>53.3 ± 13.6</td>
<td>54.9 ± 9.1</td>
<td>7.39 ± 0.05</td>
<td>35.3 ± 2.2</td>
</tr>
<tr>
<td>30 min Post ALM</td>
<td>30.4 ± 0.8</td>
<td>51.8 ± 15.6</td>
<td>55.6 ± 7.4</td>
<td>7.36 ± 0.02</td>
<td>35.1 ± 2.3</td>
</tr>
<tr>
<td>Infusion PCB</td>
<td>30.5 ± 0.5</td>
<td>50.9 ± 14.5</td>
<td>56.9 ± 8.4</td>
<td>7.37 ± 0.05</td>
<td>35.9 ± 2.4</td>
</tr>
<tr>
<td>Mid ALM</td>
<td>34.3 ± 0.4</td>
<td>56.2 ± 7.9</td>
<td>53.4 ± 5.1</td>
<td>7.42 ± 0.02</td>
<td>35.3 ± 2.2</td>
</tr>
<tr>
<td>Rewarming PCB</td>
<td>34.2 ± 0.1</td>
<td>55.5 ± 5.7</td>
<td>54.3 ± 7.4</td>
<td>7.41 ± 0.04</td>
<td>35.6 ± 2.5</td>
</tr>
<tr>
<td>End ALM</td>
<td>37.0 ± 0.0</td>
<td>52.3 ± 7.2</td>
<td>48.8 ± 6.2</td>
<td>7.46 ± 0.03</td>
<td>34.3 ± 2.8</td>
</tr>
<tr>
<td>Rewarming PCB</td>
<td>37.0 ± 0.0</td>
<td>50.4 ± 3.8</td>
<td>51.3 ± 5.9</td>
<td>7.45 ± 0.04</td>
<td>35.2 ± 1.9</td>
</tr>
</tbody>
</table>

Arterial blood gas responses to hypoxic hypothermia and rewarming comparing effects of continuous almitrine (ALM) infusion (1.0 $\mu$g·kg$^{-1}$·min$^{-1}$) to equivalent placebo (PCB) infusion. There were no significant ($p = 0.05$) differences between ALM and PCB groups. Values are means ± SD (n = 6). Esophageal temperature (Tem), arterial $O_2$ partial pressure ($P_{aO_2}$), arterial $CO_2$ partial pressure ($P_{aCO_2}$), arterial bicarbonate ion concentration ($HCO_3$).

tored using a 450-TH digital thermometer (Doric Scientific, San Diego, CA) with a series 400 rectal thermistor (Yellow Springs Instruments), Yellow Springs, OH).

Arterial and venous blood samples (1 ml) were collected in heparinized syringes at normothermia, then during cooling, hypothermia, and rewarming. Blood gases, corrected for temperature, were quantified using an ABL3 blood gas analyzer (Radiometer, Copenhagen), the microhematocrit technique (in duplicate) was used for hematocrit (Hct) measurements, hemoglobin (Hgb) was measured by the cyanmethemoglobin technique, total protein (TP) was determined by refractometry, osmolality (Osm) was quantified by freezing point depression, and albumin (Alb) was determined using a 550 Express autoanalyzer (Ciba-Corning, Medfield, MA). Percentage changes in plasma volume (%$\Delta$PV) were derived from Hct and Hgb data using the equations described by Dill and Costill (7).

Hypothermia and Rewarming Procedures

Each animal was used twice, serving as its own control. In random order, either PCB or ALM dissolved in malic acid was administered. A 1-week recovery period was given prior to the second experiment. Feed, but not water, was withheld from the evening (1700 h) prior to the day of the hypoxemia/hypothermia experiments. The animals were sedated with a low dose of sodium pentothal (3 mg·kg$^{-1}$·iv), mask-induced with isoflurane (4-5%)/compressed air mixture, intubated and maintained on 3% isoflurane anesthesia. A semi-closed breathing circuit was used with soda lime for expired CO$2$ removal. The microswine were stabilized under normothermic conditions for 20-30 min prior to taking normothermic baseline measurements and blood samples. The animals were then cooled using a temperature controlled water blanket, a hypothermic baseline was established, then either continuous iv infusion of ALM (1.0 $\mu$g·kg$^{-1}$·min$^{-1}$) or PCB at an equivalent infusion rate was administered throughout approximately 45 min of hypothermia and 3-4 h of rewarming to normothermia. Arterial hypoxemia was maintained for the entire experimental duration (Table I). After rewarming the pigs were allowed to recover. Post-experimental analgesia was provided by butorphanol tartrate (0.5 mg·kg$^{-1}$, im) to relieve any animal discomfort.

Statistics

Mean values for all experimental and calculated data at the different time intervals between control and experimental groups were compared using a two-way analysis of variance; the null hypothesis was rejected at $p < 0.05$ using a post-hoc test (Turkey's critical difference analysis).

RESULTS

Preliminary Experiments

A major consideration of this study was that the animals become hypoxic by spontaneously breathing a hypoxic gas mixture (12.1% $O_2$, 84.9% $N_2$, 3.0% isoflurane) in order to simulate hypoxic conditions encountered at an altitude of 4300 m. However, the microswine were unable to tolerate this hypoxic gas mixture under isoflurane anesthesia without undergoing respiratory and cardiovascular arrest. Thus, a compressed air gas mixture (20.3% $O_2$, 76.7% $N_2$, 3.0% isoflurane) was adapted and used in order to mimic hypoxic conditions encountered by humans at significant altitude (3000 m), without inducing respiratory collapse or severe respiratory depression.

Additional preliminary experiments designed to determine a normothermic ALM dose for anesthetized microswine ($n = 2$) demonstrated that five consecutive 100 $\mu$g·kg$^{-1}$·iv ALM doses given 3-4 min apart increased $V_\text{E}$ by approximately 30% (from an average of 3.4 L·min$^{-1}$ to 4.5 L·min$^{-1}$) by a combination of both increased RR and $V_\text{E}$. However, when either a single iv ALM dose of 150 $\mu$g·kg$^{-1}$ ($n = 1$) or three consecutive 100 $\mu$g·kg$^{-1}$·iv doses given 15 min apart ($n = 1$) were administered to hypoxic hypothermic microswine (mean Te = 28.8°C, mean $P_{aO_2} = 49$ mmHg, $n = 2$), the hypoxemia was potentiated (mean $P_{aO_2} = 32$ mmHg) and respiratory arrest ensued. These animals were resuscitated using assisted ventilation until initial rewarming, when spontaneous ventilation occurred. Continuous iv infusion of ALM at a relatively low rate (1.0 $\mu$g·kg$^{-1}$·min$^{-1}$) was used in subsequent experiments.
HYPOXIC HYPOTHERMIA SWINE MODEL—GENTILE ET AL.

### DISCUSSION

We have developed an anesthetized microswine model of hypoxemic hypothermia and rewarming for testing prophylaxes, countermeasures, and treatments. The positive ventilatory effect of ALM via activation of peripheral chemoreceptors has been well documented (15,17). We have observed a similar response to iv bolus (high dose) ALM in normothermic anesthetized microswine. However, with iv bolus infusion of ALM during hypoxemic hypothermia, respiration was depressed, hypoxemia intensified, and respiratory arrest ensued.

The anticipated positive effects of ALM on ventilation (15), as well as the augmented HPV (16,20) for increasing arterial $P_aO_2$ may have been blunted by unknown mechanisms related to hypothermia, hypoxemia, and/or anesthesia. Further, the relatively high rates of ALM administration with iv bolus infusion initially used in the present study have been shown to cause generalized pulmonary vasoconstriction, and reduce $P_aO_2$ (16). However, these investigators (16) also reported that low dose continuous ALM iv infusion (1.0 $\mu$g · kg$^{-1}$· min$^{-1}$) caused HPV thereby shunting blood to better oxygenated alveoli resulting in higher $P_aO_2$ levels. In theory, our initial results in hypoxic hypothermic microswine with iv bolus ALM administration suggested that a low dose continuous ALM iv infusion was indicated in order to minimize generalized pulmonary vasoconstriction, stimulate HPV, and potentially alleviate the hypoxemia. Nonetheless, compared to controls, continuous low dose iv infusion of ALM did not improve any measured pathophysiological responses to hypoxic hypothermia. Higher continuous iv infusion rates may remain efficacious, but these were not attempted.

In conclusion, the results from this study suggest that iv bolus infusion of ALM, in doses that stimulate ventilation under normothermic conditions, is not indicated as a field expedient treatment for hypoxic hypothermia since rapid administration may increase the incidence and intensity of the related pathophysiological consequences. Further work is required to assess the potential efficacy of different low dose continuous iv infusion regimens.

### ACKNOWLEDGMENTS

The authors wish to thank Dr. Ralph P. Francesconi for his insightful editorial contributions to this manuscript, and Servier Laboratories, Orleans, France, for their generous contribution of almitrine bimesylate.

The investigations adhered to the “Guide for the Care and Use of Laboratory Animals” prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission on life Sciences, National Research Council, (NIH Publication 86-23, 1985).

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army.

---

### TABLE II. HEMATOLOGICAL VARIABLES.

<table>
<thead>
<tr>
<th></th>
<th>$\Delta$ Tes (°C)</th>
<th>Hct (% rbc)</th>
<th>$\Delta$ Hgb (g·dL$^{-1}$)</th>
<th>$\Delta$ TP (g·dL$^{-1}$)</th>
<th>$\Delta$ Alb (g·dL$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normothemic</td>
<td>ALM 37.3 ± 0.3</td>
<td>32.8 ± 2.5</td>
<td>9.9 ± 1.0</td>
<td>6.1 ± 0.4</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>Baseline</td>
<td>PCB 37.1 ± 0.2</td>
<td>32.9 ± 2.2</td>
<td>10.0 ± 0.6</td>
<td>6.0 ± 0.4</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>Hypothermic</td>
<td>ALM 30.6 ± 0.5</td>
<td>34.4 ± 3.0</td>
<td>10.0 ± 0.9</td>
<td>6.0 ± 0.6</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>Baseline</td>
<td>PCB 30.7 ± 0.5</td>
<td>34.1 ± 3.0</td>
<td>10.1 ± 0.6</td>
<td>6.0 ± 0.4</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>30 min Post</td>
<td>ALM 30.4 ± 0.8</td>
<td>34.3 ± 2.8</td>
<td>10.0 ± 0.9</td>
<td>6.0 ± 0.4</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td>Infusion</td>
<td>PCB 30.5 ± 0.6</td>
<td>33.7 ± 3.1</td>
<td>10.2 ± 0.7</td>
<td>5.9 ± 0.5</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td>Mid</td>
<td>ALM 34.3 ± 0.4</td>
<td>32.3 ± 3.1</td>
<td>10.2 ± 0.7</td>
<td>5.7 ± 0.3</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>Rewarming</td>
<td>PCB 34.2 ± 0.1</td>
<td>33.3 ± 2.6</td>
<td>10.1 ± 0.6</td>
<td>5.7 ± 0.3</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td>End</td>
<td>ALM 37.0 ± 0.0</td>
<td>33.0 ± 2.3</td>
<td>10.0 ± 0.8</td>
<td>5.7 ± 0.5</td>
<td>3.8 ± 0.2</td>
</tr>
<tr>
<td>Rewarming</td>
<td>PCB 37.0 ± 0.0</td>
<td>33.4 ± 2.1</td>
<td>10.3 ± 0.6</td>
<td>5.8 ± 0.3</td>
<td>3.8 ± 0.3</td>
</tr>
</tbody>
</table>

Hematological responses to hypoxic hypothermia and rewarming comparing effects of continuous almitrine (ALM) infusion (1.0 $\mu$g · kg$^{-1}$· min$^{-1}$) to equivalent placebo (PCB) infusion. There were no significant ($p < 0.05$) differences between ALM and PCB groups. Values are means ± SD (n = 6). Esophageal temperature (Tes), hematocrit (Hct), hemoglobin (Hgb), total protein (TP), albumin (Alb).
REFERENCES


