Effect of Oxygen Tension on Neutrophil Mediated Killing of *Acinetobacter baumannii*

OxyBand™
the Healing Power of Oxygen

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ABSTRACT

An in vitro study was conducted to determine whether increased oxygen tension (percent oxygen provided by OxyBand™ would increase Neutrophil (PMN) mediated killing of *Acinetobacter baumannii*. OxyBand™ has been shown to increase oxygen tension (increase and maintain elevated levels of dissolved oxygen in wounds (PO2) in full thickness wounds for extended periods of time. A recent in vivo study demonstrated OxyBand™ elevated PO2 in full thickness wounds to 245 – 263 mmHg for twelve hours (when the study was completed) compared to PO2 in wounds treated with HBOT and covered with a film dressing that reached a PO2 level of 89 – 125mmHg.

Acinetobacter baumannii has emerged as a major cause of community-associated and nosocomial pneumonia. Infections with Acinetobacter have been an increasingly recognized problem for injured military personnel, particularly soldiers returning from Iraq and Afghanistan with burns and wounds caused by explosives. Studies have found in the case of *Acinetobacter baumannii* respiratory infections that neutrophils are rapidly recruited to the lungs following i.n. inoculation of the *Acinetobacter baumannii* pathogen and decline to baseline level upon clearance of the infection. Depletion of neutrophils using monoclonal antibody RB6-8C5 (in vivo) prior to infection resulted in an acute lethal infection that was associated with enhanced bacterial burdens in the lung ($P < 0.05$) and extrapulmonary dissemination to the spleen.

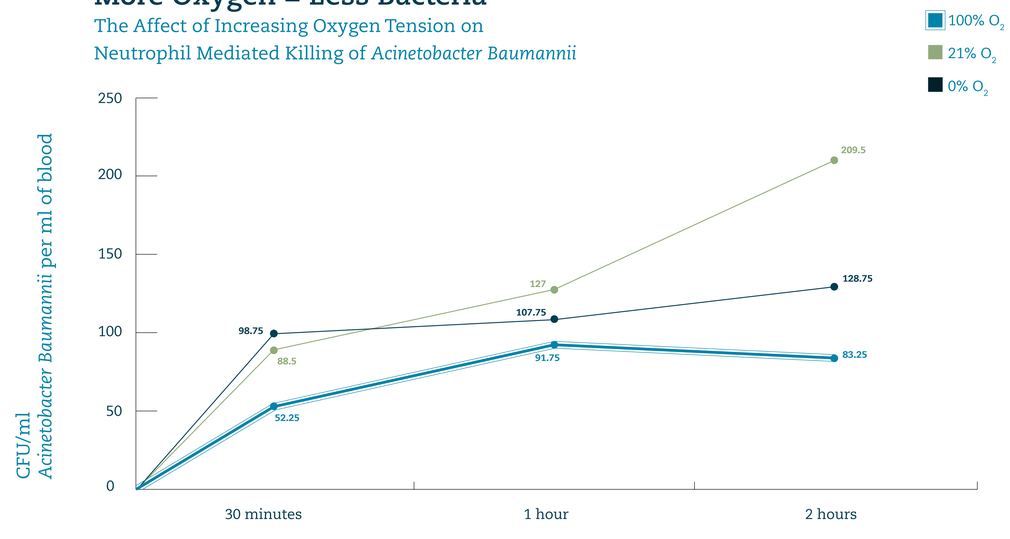
The study presented in this poster was designed to determine the affect of neutrophils on killing *Acinetobacter baumannii* by increasing oxygen tension in vitro. The question to be answered was whether killing of *Acinetobacter baumannii* by neutrophils would be proportional to local oxygen tension in vitro.

Results from the in vitro study confirm that PMNs kill *Acinetobacter baumannii* in an oxygen dependent fashion. Levels of oxygen provided by OxyBand™, should increase PMNs and therefore, increase in neutrophil mediated killing of *Acinetobacter baumannii*. Results suggest there is a beneficial effect of increasing oxygen tension on neutrophil mediated killing of *Acinetobacter baumannii*. From these data a clinical trial of traumatic and combat wounds seems indicated.

OxyBand Technologies Inc. has developed OxyBand™ – a ready to deploy topical oxygen delivery system in a dressing that requires no external oxygen source and can be stored for prolonged periods in a simple foil wrapper. The delivery system is an FDA approved (510k) device which has illustrated ability to accelerate wound healing with less pain and without infection. Preliminary studies demonstrate that OxyBand™ maintains surface oxygen levels over a wound sufficient to enhance neutrophil bacterial killing for up to 5 days. It can be manufactured in any size, including small enough to be carried in webbing or a backpack, and large enough to treat wounds over significant body surface area. OxyBand™, if beneficial in preventing or treating infection/contamination with Acinetobacter and potentially MRSA, will have a tremendous potential for widespread military use.

More Oxygen = Less Bacteria

The Affect of Increasing Oxygen Tension on Neutrophil Mediated Killing of *Acinetobacter Baumannii*



BACKGROUND

Hyperbaric oxygen has been shown to be effective in improving healing in selected clinical conditions. Similarly, local oxygen delivery has been shown to provide a stimulus to healing in a manner similar to hyperbaric oxygen. The exact mechanism of effect remains an open question for both technologies. Infections affecting wounds causing loss and death of tissue, chronicity, limbs and death represent a significant problem for both patients and the military and civilian health care delivery systems. Continued armed conflict and changing demographics of most developed societies imply that a dramatic increase in the incidence of wounds including infected and chronic wounds is growing.

OxyBand™ Studies & Demonstrated Results:

All studies (in vitro, preclinical, clinical) used the FDA approved device (510k approval number K043063). OxyBand™ is a multilayer wound dressing that keeps out water, dirt and germs, and supplies oxygen to the wound. It is designed to be applied directly over clean skin or wounds for up to 5 days. A study has shown that oxygen levels rise steadily over a wound area for the first few hours and then maintain at elevated levels through 5 days as long as the dressing remains intact and secure around the perimeter.

The present study evaluated the efficacy of oxygen in OxyBand™ on Neutrophil Mediated killing of *Acinetobacter baumannii* has been seen in wounded soldiers treated since the beginning of OIF and OEF. Multi Drug Resistant Acinetobacter and *Acinetobacter Baumannii* organisms were the most frequently recovered gram-negative isolate from war wounds and the second most frequent bacterium causing bloodstream infection in US Marines with extremity wounds during the Vietnam War.

Resistant Wound Infections

Wound infections remain the most common complication of trauma, including surgery. They lead to delayed as well as impaired healing and excess cost. Injuries in military personnel carry a particularly high risk of infection. Despite ongoing introduction of more powerful antibiotics, the rate of antibiotic resistance continues to increase. Staphylococcus aureus (MRSA) strains, *Acinetobacter baumannii* and other bacteria-demonstrating multiple antibiotic resistant are becoming epidemic. Resistance to infection for Staph aureus in particular has been shown to be dependent upon sufficient oxygen to allow phagocytic killing. Development of resistance to oxidative bacterial killing has not been described previously. *Acinetobacter baumannii*, classified as an opportunistic, Gram-negative coccobacillus although primarily considered a respiratory contaminant that may cause pneumonia or sepsis in civilian life, wound infections with acinetobacter have been an increasingly recognized problem for injured military personnel, particularly those with burns and wounds caused by explosives. Acinetobacter is naturally sensitive to a small range of antibiotics, and reports of resistance are increasing. It has been suggested that Acinetobacter is another bacterium that requires oxidative killing by neutrophils, but this impression also has not been systematically investigated.

The present study investigates the efficacy of increasing levels of oxygen present in OxyBand™ to prevent and treat Acinetobacter by examining the oxygen-dependence of neutrophil-mediated killing of *Acinetobacter baumannii*, in vitro.

The impact of increasing oxygen concentrations in infections, led OxyBand™ scientists and University of California San Francisco medical school physicians to ask whether increased oxygen tension would affect Neutrophil Mediated Killing of *Acinetobacter baumannii*. This laboratory trial was conducted at The University of California San Francisco, School of Medicine, and consisted of an in vitro study to determine whether the effect of higher oxygen tension would increase the Neutrophil Mediated killing of *Acinetobacter baumannii*. The results, are positive in vitro, and warrant testing clinically and in the field.

Study Methods

Acinetobacter baumannii were cultured and grown in a variety of growth media to optimize growth rate, viability and predictable concentration. Human neutrophils (PMN) were then isolated using the Histopaque gradient technique and their concentration, viability and bactericidal activity were determined. An apparatus was built to allow for control of temperature and oxygen concentration while mixing PMNs and bacteria for various lengths of time to measure their bactericidal activity.

After determining the growth rate of *Acinetobacter baumannii* and stationary phase concentrations in broth, the chamber was built to mix the PMNs and bacteria. This involved maintaining a humidified environment where the partial pressure of oxygen could be varied from 10mmHg through >600mmHg while maintaining a constant temperature of 37C. The chamber was configured to allow constant stirring to ensure good mixing and enable rapid gas exchange between the liquid environment of the reaction and the gas environment of the chamber. Additionally, the apparatus allowed researchers rapid access to remove bacterial samples at various time points without disturbing the environment. After the chamber was designed, built and tested, PMNs were isolated from human volunteers, and then the viability and bactericidal activity were tested. Samples were taken from the reaction at various time points and at various oxygen tensions to determine viability and the bactericidal efficacy of PMNs at those environments. After adapting the protocol for Acinetobacter the protocol was repeated 3 times.

RESULTS

Table 1 *(Selected Results)*

Table 1, shows a trend toward higher oxygen tension effecting the PMN killing of *Acinetobacter baumannii* in vitro The data shows PMNs with 100% oxygen results in a greater reduction in CFU's of Acinetobacter than with 21% oxygen and with 0% Oxygen at 30 minutes, after 1 hour and after 2 hours.

These data confirm that PMNs kill *Acinetobacter baumannii* in an oxygen dependent fashion. Results suggest there is a beneficial effect of increasing oxygen tension on neutrophil mediated killing of *Acinetobacter baumannii*. From these data a clinical trial of wounds in clinical settings and in combat situations seems indicated.

DISCUSSION

Wounds and burns remain a common experience of military personnel, whether on active duty or retired. Major injuries clearly cause significant morbidity and mortality, but the morbidity from even minor injuries may also be high, particularly if the wounds become infected. Infection of superficial wounds, particularly burn wounds, with *Acinetobacter baumannii* has become a significant problem that delays return of military personnel to active duty – or to activities of daily living. Acinetobacter is frequently resistant to multiple antibiotics. Topical antibiotics tend to impair epithelization, and thus have somewhat limited value. Topical oxygen, however, appears to enhance epithelization.

Oxygen is important in wound healing, and there is a need to find technology to deliver increased concentrations to superficial wounds. OxyBand™ as has been shown during in vitro and clinical trials to: increase PO2 and sustain increased PO2 in full thickness wounds over time; to accelerate the speed of epithelialization in partial thickness wounds compared to placebo and standard of care; reduce inflammation and decrease pain.

This investigation was initiated to determine whether increasing oxygen concentrations could enhance bacterial kill rates for *Acinetobacter baumannii* that has become important in combat infected. These in vitro results combined with prior findings about the use of topical O2 provided by OxyBand™ warrant immediate field testing.

CONCLUSION

The study presented demonstrates from a basic science standpoint that oxygen plays a critical role in resistance to infection by bacteria such as *Acinetobacter baumannii*. From a clinical standpoint, with significant military relevance, the study demonstrates that topical oxygen, OxyBand™, may be useful in treating or preventing infections by bacteria such as Acinetobacter present in traumatic and combat wounds. OxyBand™ is an easy to apply topical oxygen dressing that can deliver high levels of oxygen for up to 5 days. It requires no external oxygen source and can be stored for prolonged periods in a simple foil wrapper. Preliminary studies demonstrate that it maintains surface oxygen levels over a wound sufficient to enhance neutrophil bacterial killing for up to 5 days. It can be manufactured in any size, including small enough to be carried in a soldier's backpack, and large enough to treat burn wounds over significant body surface area. OxyBand™ may be beneficial in preventing or treating infection/contamination with Acinetobacter and MRSA, it will have a tremendous potential for widespread military use. It is small enough to replace the dressing currently supplied for all troops, and should have significantly more efficacy in healing superficial wounds (thermal and chemical burns, rope burns, scrapes, and de-gloving injuries, for example) and returning injured personnel to active duty.

REPRESENTATIVE REFERENCES

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Further References upon Request