Effect of High Oxygen Level on Antimicrobial Efficacy of Silver

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Introduction

- Silver is widely used in topical gels and impregnated dressings as an antimicrobial for healing infected and chronic wounds. In vitro studies have demonstrated varied performance of silver dressings. Many in vitro studies have compared dressings however results between studies differ. In vitro methods for silver studies have differed and outcomes measured vary, causing the comparison of results to be difficult. Little clinical evidence is available to support results of in vitro studies. Clinically silver dressing performance has also varied.

- **Silver has demonstrated minimal antimicrobial effect against common wound organisms such as Pseudomonas and E Coli in hypoxic environments until additional oxygen is added.** Bioactivation and thus antimicrobial efficacy of silver may be dependent on oxygen to convert silver into the antimicrobial cation Ag+.

- Since infected and chronic wounds are typically hypoxic, the efficacy of current treatments with silver might be improved by increasing the oxygen level in the wound. OxyBand Wound Dressing has been shown to increase PO_2 in wounds. The present study was conducted to determine whether additional oxygen (the % oxygen in OxyBand) improves the antimicrobial efficacy of silver (silver nitrate $AgNO_3$) on two clinical isolates, Pseudomonas aeruginosa and **methicillin-resistant *Staphylococcus aureus*** (MRSA) both common in wounds.

Methods

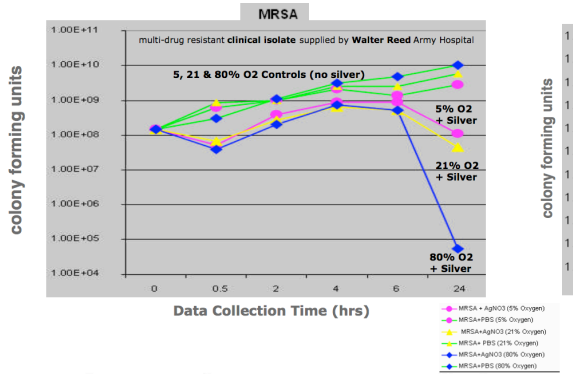
- Cultures of two isolates were grown on TSA+ blood (TSAB) plates. Plated cultures were transferred into flasks of 100 ml TSA broth until cell density approximately matching a 0.5 McFarland standard, ~ 108 CFU/ml of cells. Five ml aliquots test tubes were treated with either $AgNO_3$ (0.5% final conc.) or sterile PBS and placed into vented canisters with free flowing 5:90:5, 21:74:5, or 80:15:5 (O2:N2:CO2) gas (5%, 21%, 80% O2). Canisters were placed into 37°C orbital shaking incubators set to 135 RPM. At 0.5, 2, 4, 6, and 24 hrs, 0.5 ml of the cultures were centrifuged, and resuspended in 0.85% sodium chloride, 1% polysorbate 20, and 0.1% sodium thioglycolate solutions and cultures were drop-dilution plated (5 replicate 10 µl drops/ dilution) to determine the effect of the AgNOB3B and OB2. Cultures were assayed for ATP using an Invitrogen ATP, luciferin-luciferace kit to determine differences in cellular ATP concentrations, assayed suspension fluid/medium, pre-lysed cells from all cultures for Ambient ATP concentration and lysis solution were lysed to determine total ATP concentration.

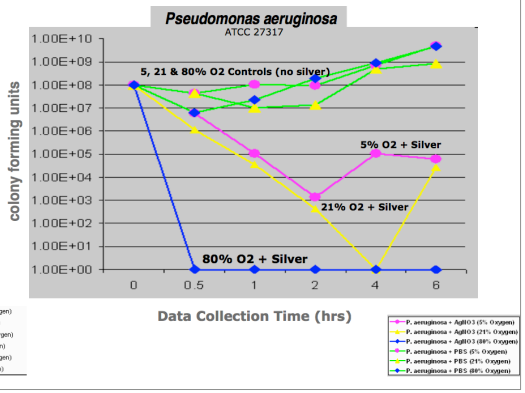
Results

- The graphs show inhibition of MRSA and Pseudomonas isolates treated with Silver (0.5% $AgNO_3$) and 80% Oxygen compared to 0.5% $AgNO_3$ and 5% and 21% Oxygen and 50µl 1x PBS and varying concentrations of oxygen. Results are an average of about five replicates 10 ml drops of $AgNO_3$ or PBS treated culture.

- *For MRSA treated with Silver Nitrate and 80% Oxygen there was a* > 5-log drop when compared to the growth of the PBS-treated cells by the end of 24 hrs, with most of the cell reduction occurring in the latter 19 hrs compared to the Silver Nitrate and 5% and 21% Oxygen that exhibited a 1 log difference.

- For Pseudomonas treated with Silver Nitrate and 80% Oxygen there was an immediate (within 30 minutes) and a sustained kill of the Pseudomonas compared to the Pseudomonas treated with Silver Nitrate and 5% Oxygen, the Pseudomonas was not killed and with 21% Oxygen the Pseudomonas came back or quiescent and began to come back at 4 hours.





Conclusion

- The results of the present study demonstrate 80% Oxygen combined with Silver (Silver Nitrate) is more effective as an antimicrobial against MRSA and Pseudomonas in vitro, than Silver (Silver Nitrate) at either hypoxic (5%) or ambient air (21%) levels of Oxygen. These results suggest there is an additive effect of higher levels of Oxygen to Silver Nitrate in treating MRSA and Pseudomonas. The clinical significance of these results could be very important in the treatment of infected and chronic wounds which are often hypoxic. The results suggest a combination treatment of Silver and high levels of oxygen may perform better as an antimicrobial than silver alone. More studies are needed to determine clinical results and with other wound pathogens.