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Hyperbaric hyperoxia suppresses growth of *Staphylococcus aureus*, including methicillin-resistant strains

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Abstract
Purpose. We investigated the effects of increased oxygen tension on the in vitro growth of *Staphylococcus aureus* (MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA), and *Escherichia coli* (*E. coli*).
Methods. The effects of oxygen tension [normobaric normoxia (21% O_2 at 1 atm), normobaric hyperoxia (100% O_2 at 1 atm), hyperbaric normoxia (21% O_2 at 2 atm), and hyperbaric hyperoxia (100% O_2 at 2 atm) on the in vitro growth of MRSA, MSSA, and *E. coli* were investigated by population analysis.
Results. Compared with normobaric normoxia, a 90-min exposure to hyperbaric hyperoxia significantly inhibited growth of both MRSA (by 25.0 ± 3.0%, mean ± SEM; $P < 0.01$) and MSSA (by 24.0 ± 3.3%; $P < 0.01$). Normobaric hyperoxia and hyperbaric normoxia were without effect. In contrast, the growth of *E. coli* was not affected by any of the above treatments.
Conclusion. Our results indicate that the bacterium *S. aureus*, including resistant strains, is susceptible to oxygen stress. The observation that relatively brief (90-min) treatment with hyperbaric hyperoxia is sufficient to produce significant growth inhibition suggests that hyperbaric hyperoxia may be useful in the treatment of serious staphylococcal infections.

Key words Methicillin-resistant *Staphylococcus aureus* · Methicillin-sensitive *Staphylococcus aureus* · *Escherichia coli* · Hyperbaric oxygen

Introduction

Life-threatening infections with *Staphylococcus aureus*, particularly those with the methicillin-resistant strain (MRSA), continue to be a common problem in medical and surgical intensive care unit patients [1]. MRSA, which may represent up to 60% of *S. aureus* isolates in some series, is resistant to β-lactam-containing antimicrobials, and vancomycin is currently the antibiotic of last resort [1]. Recently, however, clinical isolates of *Staphylococcus* strains with reduced sensitivity to vancomycin have been reported [2]. The emergence of these vancomycin-resistant *S. aureus* (VRSA) strains will undoubtedly pose a particular challenge, since clinically effective antimicrobial treatment will be even more limited. Other treatment modalities and adjuvants will thus need to be considered.

Anaerobic bacteria such as *Clostridium* strains lack defenses against oxidants, and multiplication of these organisms can be inhibited by increasing oxygen tension [3]. Indeed, hyperbaric oxygen (HBO) has been reported to increase tissue Po_2 to levels that significantly affect anaerobic bacterial growth and to provide clinical benefits in the treatment of such infections [3,4]. In contrast, aerobic bacteria have higher levels of endogenous antioxidants, and these bacteria are generally reported to be resistant to hyperoxia [5]. Some reports, however, suggest that prolonged, intense exposure to HBO may be bacteriostatic for certain facultative aerobic bacteria, including *Escherichia coli* [6], *Enterobacteriaceae* [7], and *Streptococcus faecalis* [8]. Moreover, HBO has been reported to decrease infections in thermal burn wounds [9], osteomyelitis [10], and diabetic wounds [11]. In addition to its obvious potential in the treatment of wound infections, HBO therapy may also provide clinical benefit in the control of other infections with aerobic organisms [11].

Although previous studies have suggested that HBO may also affect aerobic bacterial metabolism [5,6], the effect of HBO on the viability of MRSA remains unknown. Further investigation, therefore, is required to elucidate the sensitivity of this organism to high oxygen. This study was undertaken to evaluate the effect of HBO on the in vitro proliferation of both MRSA and methicillin-sensitive *S. aureus* (MSSA), as well as that of the common strain of *E. coli*. An HBO exposure

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protocol similar to that used in human trials was used and compared with the effect of normobaric treatment.

Materials and methods

Experimental samples

Fresh clinical bacterial isolates of MRSA (24 patients), MSSA (20 patients), and *E. coli* (16 patients) were recovered from the surgical wounds or peripheral blood of patients admitted to the hospital. Isolates were used without any additional passage in vitro, to avoid altering the biological nature of the bacteria. Microbial suspensions were prepared by loop inoculation of colonies from an overnight plate culture into a tissue culture medium (Eagle medium; Difco, Detroit, MI, USA), followed by serial dilution, to give colony counts within the range 100–500. After overnight incubation in trypticase soy agar medium, a single colony of each bacterium was transferred to a sterile tube containing 10 ml of Eagle's medium and then diluted four times.

Test conditions

To determine the effect of HBO on bacterial growth, a simplified method of traditional population analysis was used: a 1.0-ml aliquot of the diluted suspension was transferred aseptically to four sterile Petri dishes (3.5 cm in diameter), and each of the four Petri dishes containing the bacterial suspension was assigned to one of the four treatment groups: hyperbaric hyperoxia (2.0 atm, 100% O_2), normobaric hyperoxia (1.0 atm, 100% O_2), hyperbaric normoxia (2.0 atm, 21% O_2), and normobaric normoxia (1.0 atm, 21% O_2) (control group). The dishes in the normobaric groups were placed in chambers (height, 40 cm; width, 40 cm) at ambient pressure perfused at a continuous flow rate of 10 l·min^{-1} either with air (normobaric normoxia group) or with 100% O_2 (normobaric hyperoxia group). Dishes in the hyperbaric groups were placed in a sealed chamber at 2 atm HBO (Hanimatsu-Tekkou, Tokyo, Japan) with either 21% O_2 (hyperbaric normoxia group) or 100% O_2 (hyperbaric hyperoxia group) as the pressurizing gas. Immediately following the 90-min exposure period, a 0.1-ml aliquot of each bacterial suspension was spread using a glass rod onto a trypticase soy agar plate and incubated at 37°C. The number of colonies (CFU, colony-forming units) present at 16 h and 24 h was then determined.

Statistical analysis

Values are expressed as means ± SEM. Statistical analysis was performed by using either a two-factor analysis of variance followed by an unpaired *t*-test or a repeated-measures analysis of variance followed by a paired *t*-test. *P* values less than 0.05 were considered significant.

Results

A typical example of MRSA growth after a 90-min exposure to hyperbaric hyperoxia is shown in Fig. 1. The absolute numbers of CFU under control conditions (90-min exposures to normobaric normoxia) were 240.0 ± 24.3 CFU/plate in the MRSA group, 177.1 ± 13.3 CFU/plate in the MSSA group, and 242.0 ± 35.1 CFU/plate in the *E. coli* group (mean 24-h test growth ± SEM). The effects of the various experimental conditions on the 24-h growth of each strain were expressed in percentage terms relative to the number of CFU present at the same time point in the control group (i.e., normobaric normoxia) (Table 1). Compared with control, a 90-min exposure to hyperbaric hyperoxia significantly inhibited growth of both MRSA (by 25.0 ± 3.0%, mean ± SEM; $P < 0.01$) and MSSA (by 24.0 ± 3.3%; $P < 0.01$), although normobaric hyperoxia and

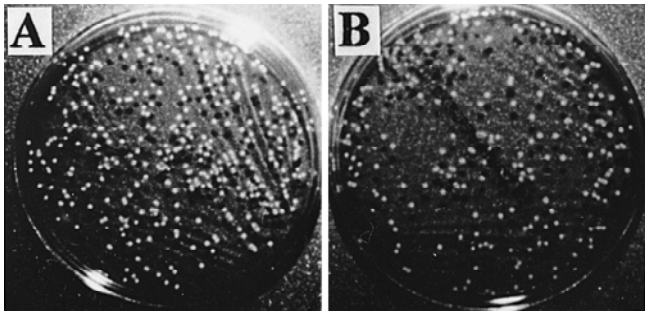

Fig. 1. Typical colony-forming units (CFU) of methicillin-resistant *Staphylococcus aureus* (MRSA) in culture plates 24 h after a 90-min exposure to (**A**) normobaric normoxia (control, 1 atm, 21% O_2) or (**B**) hyperbaric hyperoxia (2 atm, 100% O_2)

Table 1. Percent reduction of bacterial growth by various treatments

Bacteria	Normobaric hyperoxia	Hyperbaric normoxia	Hyperbaric hyperoxia
MRSA	0.1 ± 1.7	4.0 ± 2.6	25.0 ± 3.1[a,b,c]
MSSA	1.0 ± 1.3	4.6 ± 1.8	24.0 ± 3.3[a,b,c]
Escherichia coli	0.1 ± 3.0	2.4 ± 8.4	8.0 ± 8.8

Effect of normobaric hyperoxia (1 atm, 100% O_2), Hyperbaric normoxia (2 atm, 21% O_2), and Hyperbaric hyperoxia (2 atm, 100% O_2) on bacterial growth. Each result is shown as a percentage of the number of CFU in the control (normobaric normoxia: 1 atm, 21% O_2). Values are expressed as mean ± SEM. [a] $P < 0.01$ vs normobaric normoxia; [b] $P < 0.01$ vs normobaric hyperoxia; [c] $P < 0.01$ vs hyperbaric normoxia. MRSA, Methicillin-resistant *Staphylococcus aureus*; MSSA, Methicillin-sensitive *S. aureus*

hyperbaric normoxia were without effect. The growth response of *E. coli* was not affected by any of the above treatments.

Discussion

Our results indicate that hyperbaric hyperoxia produces a significant reduction in the growth of both MRSA and MSSA. The observation that a significant effect can be produced by a single relatively brief (90-min) exposure to HBO—of an intensity and duration that could easily be achieved in treating human patients—suggests that HBO therapy has potential as a treatment for serious *Staphylococcus* infections, including those involving resistant strains.

Our findings are supported by other reports in which high oxygen stresses altered cellular morphology, colony appearance, and growth characteristics in *S. aureus* [12,13]. This potential benefit of HBO therapy may prove to be particularly important in the treatment of patients infected with the newly emergent vancomycin-resistant strains, where antimicrobial options are currently very limited. This idea clearly requires further testing.

In our study, hyperbaric hyperoxia proved to exert a suppressant effect on the growth of MRSA and MSSA strains. It appears, therefore, that *S. aureus* strains, including MRSA, can be included among those microorgamisms whose protection against hyperoxic toxicity is limited. Theoretically, the Po_2 in the chamber during hyperbaric hyperoxia can reach approximately 1500 torr, whereas in normobaric hyperoxia it reaches approximately 700 torr, and in hyperbaric normoxia it is approximately 300 torr. Since the growth of both strains was unaffected by either normobaric hyperoxia or hyperbaric normoxia, HBO with an oxygen concentration >700 torr is needed to suppress the growth of such strains. This is in accord with the finding of Irvin et al. that a prolonged exposure to hyperbaric normoxia (2 atm, 21% O_2, 6 h) did not produce any significant effect on the growth of *S. aureus* [14]. Although the tissue levels of oxygen are uncertain, these results suggest that HBO is necessary to achieve the Po_2 sufficient to have a clinical effect.

As indicated in Fig. 2, most investigators believe that hyperoxic toxicity is due to the production of reactive oxygen species, such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot OH$), at a rate in excess of the capacity of the cellular defense mechanisms to inactivate such species [15,16]. It has been reported that superoxide anion is relatively unreactive, but it is considered dangerous because its dismutation results in the formation of H_2O_2, which can potentially generate the highly reactive $\cdot OH$ radical in

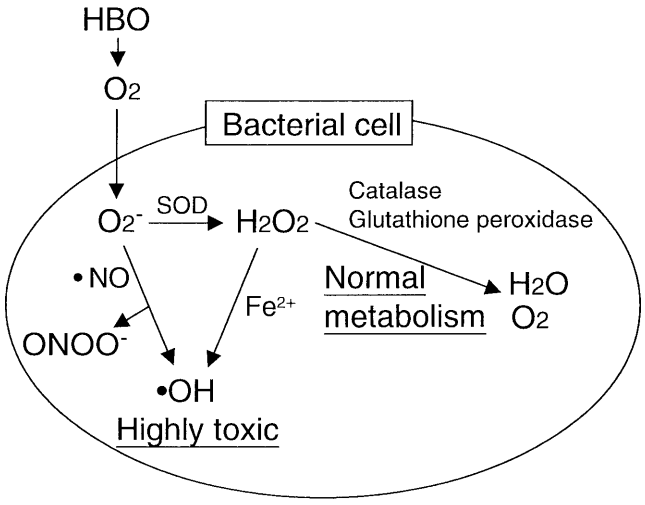

Fig. 2. Free radical generation induced by hyperbaric oxygen (HBO) in bacterial cells. *SOD*, Superoxide dismutase

the presence of transition metal ions [17]. In bacteria, such species cause DNA strand breaks, degradation of RNA, inhibition of amino acid biosynthesis, and inactivation of membrane transport proteins [5]. Intracellular antioxidants (superoxide dismutase [SOD], catalase, or glutathione peroxidase) are considered important in the protection of aerobes against oxidant damage, and increased tolerance to oxidant stress is associated with the induction of these enzymes.

A 90-min exposure to HBO did not significantly inhibit the growth of *E. coli* strains. Although hyperbaric hyperoxia seemed to result in slight decreases in the growth of *E. coli*, the difference from the control group failed to reach statistical significance. Hence, these results indicate that the susceptibility of aerobes to oxygen tension varies significantly among the different species. Indeed, it has been reported that the amount of endogenous SOD increases dramatically during high oxygen stress in *E. coli* cells [17]. The increased capacity of *E. coli* to detoxify reactive oxygen species may thus attenuate the bacterio-cidal effect of elevated oxygen tension (at least when a 90-min exposure protocol is used).

Injury and infection, as well as various pathological conditions, can markedly decrease tissue Po_2, whereas exposure to HBO (100% O_2 at 2.4 atm) increases the tissue Po_2 in wounds to levels above 1000 mmHg [18]. HBO has been reported to increase the phagocytic killing ability of polymorphonuclear leukocytes, presumably by promoting the production of reactive oxygen species [19], whereas lymphocyte proliferation is decreased [20]. HBO also promotes the production of collagen by fibroblasts [21], thereby allowing wound healing in hypoxic tissues. In addition, beneficial results with HBO have been reported in various animal models

of bacterial sepsis [22,23] and zymosan-induced shock [24]. Interestingly, patients suffering from MRSA osteomyelitis have been successfully treated by additional surgical debridement, antibiotics, and adjunctive HBO [10]. Most likely, these treatments all work synergistically, with a significant contribution from the direct effect of HBO on bacterial growth reported here.

To be effective, hyperbaric oxygen may be inhaled through a mask or an endotracheal tube in a large, multioccupant chamber. When used according to standard protocols, with oxygen pressure not exceeding 3 atm and treatment sessions limited to a maximum of 120 min, HBO therapy is of proven safety [11]. However, critically ill patients who have required a high concentration of normobaric oxygen for a prolonged period and who then undergo repeated exposure to HBO are at greater risk of toxic pulmonary effects [11]. The decision whether to employ HBO against infections producing septic shock may need to be based on the clinical state of the patient.

In summary, the effects of oxygen tension on the in vitro growth of MRSA, MSSA, and *E. coli* isolated from patients with infections were investigated by population analysis. A 90-min exposure to hyperbaric hyperoxia, but not to hyperbaric normoxia, significantly reduced 24-h growth in both the MRSA and the MSSA groups. These results suggest that exposure to hyperbaric hyperoxia, at an intensity and duration that could easily be achieved in treating human patients, has a potentially important inhibitory effect on the growth of *Staphylococcus* strains, including methicillin-resistant strains. Thus, hyperbaric hyperoxia in combination with antibiotic treatment should provide a better bacterostatic effect than that seen with antibiotic therapy alone. Additional data will be needed to fully assess the clinical implications of these findings.

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References

1. Casewell MW (1995) New threats to the control of methicillin-resistant *Staphylococcus aureus*. J Hosp Infect 30:465–471
2. Hiramatu K, Aritaka N, Hanaki H, Kawasaki H, Hosoda Y, Hori S, Fukuchi Y, Kobayashi I (1997) Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. Lancet 350:1670–1673
3. Walden WC, Hentges DJ (1971) Differential effects of oxygen and oxidation-reduction potential on the multiplication of three species of anaerobic intestinal bacteria. Appl Microbiol 30:781–785
4. Riseman JA, Ziser WA, Curtis A, Graham DR, Konrad HR, Ross DS (1990) Hyperbaric oxygen therapy for necrotizing fasciitis reduces mortality and the need for debridements. Surgery 108:847–850
5. Park MK, Myers RA, Maezella L (1992) Oxygen tensions and infection: modulation of microbial growth, activity of antimicrobial agents, and immunologic responses. Clin Infect Dis 14:720–740
6. Brown OR (1972) Reversible inhibition of respiration of *Escherichia coli* by hyperoxia. Microbios 5:7–16
7. Bornside GH, Pakman LM, Ordoñez AA Jr (1975) Inhibition of pathogenic enteric bacteria by hyperbaric oxygen: enhanced antibacterial activity in the absence of carbon dioxide. Antimicrob Agents Chemother 7:682–687
8. Gottlieb SF, Solosky JA, Aubrey R, Nedelkoff DD (1974) Synergistic action of increased oxygen tensions and PABA-folic acid antagonists on bacterial growth. Aerosp Med 45:829–833
9. Hart GB, O'Reilly RR, Broussard ND, Cave RH, Goodman DB, Yanda RL (1974) Treatment of burns with hyperbaric oxygen. Surg Gynecol Obstet 139:693–696
10. Sheftel TG, Mader JT, Pennick JJ, Cierny G III (1985) Methicillin-resistant *Staphylococcus aureus* osteomyelitis. Clin Orthop 198:231–239
11. Tibbles PM, Edelsberg JS (1996) Hyperbaric-oxygen therapy. N Engl J Med 334:1642–1647
12. Ollodart R, Blair E (1965) High pressure oxygen as an adjunct in experimental bacteremic shock. JAMA 191:736–741
13. Schmidt JP, Ball RJ (1967) Effect of high oxygen concentration on virulence of *Staphylococcus aureus*. Appl Microbiol 15:757–758
14. Irvin TT, Suwanagul A, Norman JN, Smith G (1967) The effects of hyperbaric oxygen on *Staphylococcus aureus*. Surg Gynecol Obstet 125:1217–1231
15. Fridovich I (1983) Superoxide radical: an endogenous toxicant. Annu Rev Pharmacol Toxicol 23:239–257
16. Jamison D, Chance B, Cadenas E, Boveris A (1986) The relation of free radical production to hyperoxia. Annu Rev Physiol 46:703–719
17. Gregory EM, Fridovich I (1973) Induction of superoxide dismutase by molecular oxygen. J Bacteriol 114:543–548
18. Nyman S, Lindhe J, Zederfeldt B (1971) Granulation tissue formation and respiratory gas tensions in wound fluid in estradiol and progesterone treated female rabbits. Acta Chir Scand 137: 703–707
19. Mader JT, Brown GL, Guckian JC, Reinarz JA (1980) A mechanism for the amelioration by hyperbaric oxygen of experimental staphylococcus osteomyelitis in rabbits. J Infect Dis 142: 915–921
20. Gadd MA, McClellan DS, Neuman TS, Hansbrough JF (1990) Effect of hyperbaric oxygen on murine neutrophil and T-lymphocyte function. Crit Care Med 18:974–979
21. Juva K, Prockop J, Cooper GW (1966) Hydroxylation of proline and the intracellular accumulation of a polypeptide precursor of collagen. Science 152:92–96
22. Thom SR, Lauermann MW, Hart GB (1986) Intermittent hyperbaric oxygen therapy for reduction of mortality in experimental polymicrobial sepsis. J Infect Dis 154:504–510
23. Muhvich KH, Myers RAM, Marzella L (1988) Effect of hyperbaric oxygenation, combined with antimicrobial agents and surgery, in a rat model of intraabdominal infection. J Infect Dis 157:1058–1061
24. Luongo C, Imperatore F, Cuzzocrea S, Filippelli A, Scafuro MA, Mangoni G, Portoland F, Rossi F (1998) Effects of hyperbaric oxygen on a zymosan-induced shock model. Crit Care Med 26:1972–1976