Contents lists available at Wolters Kluwer - Medknow

Medical Gas Research

journal homepage: www.medgasres.com

Synergistic application of antibiotics and hyperbaric oxygen therapy

Dittmar Chmelař, Ondřej Jor, Jakub Tlapák, Michal Hájek*

The synergistic application of antibiotics and hyperbaric oxygen therapy (HBOT) presents a promising avenue to improve bacterial eradication, particularly against resistant strains and biofilm-associated infections. HBOT involves administering 100% oxygen at elevated pressures, thereby increasing tissue oxygenation and potentially increasing the efficacy of antimicrobial agents.

Mechanisms of enhancement of antibacterial activity: Several studies have elucidated the mechanisms by which HBOT enhances antibiotic action.

Increased oxygenation: HBOT raises tissue oxygen levels, which may amplify the bactericidal properties of certain antibiotics, particularly those that depend on oxygen-related mechanisms. Wild strains of *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*, and *Proteus mirabilis* were evaluated in an experimental hyperbaric chamber to examine the potential influence of hyperbaric oxygen on their susceptibility to antibiotics, including ampicillin, ampicillin combined with sulbactam, cefazolin, cefuroxime, cefoxitin, gentamicin, sulfamethoxazole combined with trimethoprim, colistin, oxolinic acid, ofloxacin, tetracycline, and aztreonam. Ninety-six-well microplates inoculated with these bacteria and antibiotics in Mueller-Hinton broth were incubated for 24 hours under standard atmospheric conditions (normobaric normoxia) or within an experimental hyperbaric chamber (HAUX, Germany) at 2.8 ATA of 100% oxygen (hyperbaric hyperoxia). In the case of *Pseudomonas aeruginosa*, the impact of hyperbaric oxygen on antibiotic susceptibility could not be assessed, as bacterial growth was completely inhibited under these conditions. Further testing with wild strains of pseudomonads, burkholderias, and stenotrophomonads confirmed that these bacteria ceased growing under hyperbaric conditions at 2.8 ATA of 100% oxygen, and also demonstrated that this growth inhibition was reversible.[1]

Elevated oxygen levels can also trigger metabolic and genetic changes in microorganisms, such as increased transmembrane potential, reduced protein synthesis, and the activation of antioxidant defenses, which may modify their susceptibility to antimicrobial agents. Additionally, oxygen concentration can influence the pharmacokinetics of an antimicrobial agent by affecting systemic hemodynamics and localized blood circulation. The first two mechanisms play a more significant role in enhancing the antimicrobial activity of specific drugs.[2]

The susceptibility of various bacteria to different antibiotics was also assessed under hyperbaric conditions simulating saturation diving. The impact of hyperbaric helium and oxygen (heliox) on antibiotic stability and β-lactamase induction was examined. *Escherichia coli* and *Salmonella typhimurium* displayed increased resistance to penicillin (up to 23%), gentamicin (up to 46%), and rifampicin (up to 18%) at pressures of 36 and 71 ATA. Exposure to 71 ATA of heliox did not compromise antibiotic efficacy but led to heightened β-lactamase production in inducible strains of *Staphylococcus aureus* and *Bacillus subtilis*, as well as increased β-galactosidase production in inducible *Escherichia coli*. This enhanced resistance to antibiotics under saturation diving conditions may, in some instances, be attributed to effects of hyperbaric pressure on bacterial induction mechanisms. The experimental setup developed in this study allows for a more detailed investigation into the impact of hyperbaric stress on antibiotic resistance and bacterial response mechanisms.[3]

The growth of *Staphylococcus aureus* ATCC 6538P was analyzed in stationary broth cultures (11 mm deep) subjected to hyperbaric oxygen (100% O_2 at 3.0 ATA). The minimum inhibitory concentration of penicillin, streptomycin, tetracycline, oxytetracycline, kanamycin, and cephalothin was measured after exposure to hyperoxia for 3, 6, and 12 hours. Logarithmic bacterial growth was delayed by 60% under hyperoxic conditions, whereas exposure to air at 3.0 ATA did not affect growth. The longer the exposure to hyperoxia, the lower the minimum inhibitory concentration. Regardless of the antibiotic used, minimum inhibitory concentration values for unexposed control samples were 73% after 3 hours of hyperoxia, 53% after 6 hours, and 34% after 12 hours. Similar findings with hyperoxia and an iodophor suggest a general enhancement of antibacterial efficacy. Although hyperoxia alone primarily exhibits bacteriostatic effects, its combination with antibiotics may provide an effective therapeutic approach for bacterial infections.[4]

Biofilm disruption: Bacterial biofilms exhibit heightened resistance to antibiotics. HBOT has been shown to compromise biofilm integrity, thereby increasing bacterial susceptibility to antimicrobial treatments. The simulated effect of ciprofloxacin therapy was examined in a *Pseudomonas aeruginosa* PAO1 biofilms model incorporating agarose aggregates in conjunction with hyperbaric oxygen treatment. The killing effect of ciprofloxacin was modeled using parameters derived from existing literature along with newly estimated values.[5] Microrespirometry experiments measured oxygen consumption in the PAO1 strain, with parameters validated against previous HBOT data from Kolpen et al.[6] The oxygen model accurately predicted oxygen concentrations over time and at different biofilm depths. At 2.8 ATA, HBOT increased oxygen penetration depth nearly fourfold, consistent with theoretical predictions based on stationary diffusion-consumption balance. The full reaction-diffusion model revealed that HBOT significantly enhanced bactericidal effects of ciprofloxacin on PAO1 biofilms, aligning with experimental findings from Kolpen et al.[6,7] This increased bacterial eradication led to reduced oxygen consumption in the outer biofilm layers, allowing for deeper oxygen penetration and further enhancing antimicrobial activity.

Enhanced immune response: Improved oxygenation through HBOT can strengthen the immune system, aiding in bacterial clearance. Infectious endocarditis (IE) is a heterogeneous condition varying in severity and often necessitating surgical intervention. The in-hospital mortality rate ranges from 15% to 20%. While HBOT has been considered an adjunct therapy for severe bacterial infections for decades, its clinical application remains limited due to accessibility constraints and a lack of well-designed studies. This review highlights potential benefits of HBOT in treating IE, emphasizing its effects on host response, tissue hypoxia, biofilms, antibiotics, and pathogens. Although preclinical animal studies have demonstrated effectiveness of HBOT in treating IE, no clinical trials have yet assessed its feasibility in human patients. As new therapeutic approaches for IE are urgently needed, adjunctive HBOT could serve as a valuable option for certain patients to reduce morbidity, mortality, and long-term complications.[8]

Bacteria have evolved different mechanisms of resistance to oxidative stress over the course of evolution. Catalase, superoxide dismutase, and peroxidases degrade reactive oxygen species (ROS) and protect bacterial cells.[9] DNA repair mechanisms: e.g., RecA or MutY are involved in the repair of ROS-induced DNA damage.[10] Stress regulons: e.g., OxyR and SoxRS regulate genes responding to oxidative stress.[11]

Amplification of the immune response (e.g., pharmacological stimulation of ROS) may lead to increased pressure on bacteria that selects for more resistant strains. This creates a paradox – stronger immunity may promote the emergence of bacteria capable of more efficient survival.

Excessive ROS release may lead to the selection of bacterial strains with higher antioxidant capacity or mutations in regulators of stress responses.[12] Some bacteria (e.g., *Staphylococcus aureus*) can survive in neutrophils due to their ability to detoxify ROS and modify metabolism.[13] The interaction between the host immune response and bacterial resistance is dynamic and bidirectional. While the enhancement of neutrophil function (especially ROS production) is effective in controlling infection, it can simultaneously lead to the selection of bacterial strains with higher resistance. Effective therapies should take this balance into account and seek not only to enhance immunity but also to target the specific mechanisms by which bacteria defend against this immunity. The topic of the relationship between oxygen concentration

and the permeability of the outer membrane of bacteria, especially in relation to the expression of porins, is very specific and not yet fully clarified. Studies in this area show that aerobic versus anaerobic conditions can affect the expression of porins such as outer membrane protein F (OmpF) and outer membrane protein C (OmpC), which in turn has an impact on the permeability of the outer membrane and thus on the susceptibility of bacteria to antibiotics. Proteomics plays a crucial role in this field to quantify and analyze expression changes. The direct relationship between oxygen concentration and porin expression has not yet been fully elucidated. Under anaerobic conditions, decreased OmpF expression and sometimes increased OmpC expression are generally observed.[14] The regulation is partly via the EnvZ/OmpR system, but oxygen can also affect the FNR (fumarate and nitrate reductase regulator), which is active under anaerobic conditions and alters the expression of a number of genes including membrane proteins.[15] This leads to a change in membrane permeability-bacteria can defend themselves against harmful substances (including antibiotics) in low-oxygen environments (e.g., biofilms or gut).

Modern proteomic methods such as LC-MS/MS (liquid chromatography-tandem mass spectrometry) allow the quantification of changes in OmpF and OmpC expression under different conditions. Membrane fraction of bacteria is isolated. This is followed by enzymatic cleavage of proteins (e.g., by trypsin), and then identification and quantification by mass spectrometry. A previous study shows that, for example, during hypoxia, the detectability of OmpF in the outer membrane is reduced, while other proteins (e.g., iron transporters) can be upregulated. Altered porin expression affects the uptake of antibiotics such as β-lactams and fluoroquinolones.

Clinical implications and applications: The combination of antibiotics and HBOT has shown promise in various clinical settings.

Necrotizing soft tissue infections: For life-threatening infections such as necrotizing fasciitis, HBOT has been utilized to enhance antibiotic efficacy, mitigate tissue hypoxia, and suppress anaerobic bacterial growth. A meta-analysis was conducted to assess the effectiveness of HBOT in treating necrotizing soft tissue infections. Data sources included PubMed, Embase, Web of Science, the Cochrane Library, and reference lists. The study analyzed observational trials comparing HBOT with standard care or non-HBOT treatments. The primary outcome was mortality rate, while secondary outcomes included debridement frequency, amputation rate, and complication incidence. Among 49,152 patients, 1448 received HBOT, while 47,704 served as controls. The mortality rate was significantly lower in the HBOT group compared to the non-HBOT group [relative risk (*RR*) = 0.522, 95% confidence interval (*CI*) (0.403, 0.677), $P < 0.05$]. However, the HBOT group underwent more debridements than the control group [standardized mean difference (*SMD*) = 0.611, 95% *CI* (0.012, 1.211), $P < 0.05$]. No significant difference was observed in amputation rates [*RR* = 0.836, 95 % *CI* (0.619, 1.129), $P > 0.05$]. Furthermore, the incidence of multiple organ dysfunction syndrome was lower in the HBOT group [*RR* = 0.205, 95% *CI* (0.164, 0.256), $P < 0.05$]. Current evidence supports the role of HBOT in significantly reducing mortality and complications in necrotizing soft tissue infections patients.[16]

Considerations and future directions: Integrating HBOT with antibiotic therapy presents a promising strategy for enhancing bacterial eradication, particularly in difficult-to-treat infections. Continued research and clinical trials are essential to fully understand this combined approach and develop standardized treatment protocols. In conclusion, the integration of HBOT with antibiotic treatment offers a compelling strategy to enhance bacterial eradication, particularly in challenging infections. Ongoing research and clinical trials are vital to fully elucidate the potential of this combined approach and establish standardized treatment protocols (**Figure 1**).

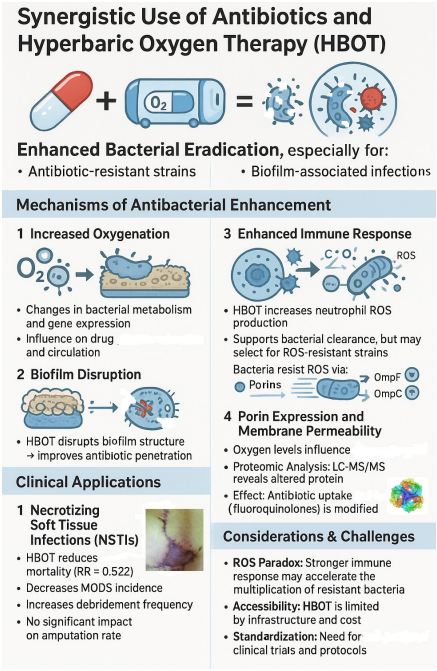

Figure 1 | Mechanisms of enhancement of antimicrobial activity.
Created with OpenAI ChatGPT. The final content was verified and edited by the authors. LC-MS/MS: Liquid chromatography–tandem mass spectrometry/mass spectrometry; MODS: multiple organ dysfunction syndrome; OmpC: outer membrane protein C; OmpF: outer membraneprotein F; ROS: reactive oxygen species; RR: relative risk.

Dittmar Chmelař, Ondřej Jor, Jakub Tlapák, Michal Hájek*
Institute of Laboratory Medicine, Institute of Microbiology, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic (Chmelař D, Hájek M)
Centre for Hyperbaric Medicine of Faculty of Medicine University of Ostrava and Ostrava City Hospital, Ostrava, Czech Republic (Chmelař D, Jor O, Hájek M)
Centre of Hyperbaric Medicine, Ostrava City Hospital, Ostrava, Czech Republic (Jor O, Hájek M)
Department of Anaesthesiology, Resuscitation and Intensive Care Medicine, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic (Jor O)
The Institute of Aviation Medicine, Prague, Czech Republic (Tlapák J)
Faculty of Biomedical Engineering, Czech Technical University in Prague, Czech Republic (Tlapák J)
***Correspondence to:** Michal Hájek, PhD, michalhajek@email.cz or michal.hajek@osu.cz.
https://orcid.org/0000-0003-1017-8470
(Michal Hájek)

Date of submission: March 25, 2025
Date of decision: June 3, 2025
Date of acceptance: June 9, 2025
Date of web publication: August 18, 2025

https://doi.org/10.4103/mgr.MEDGASRES-D-25-00086
How to cite this article: *Chmelař D, Jor O, Tlapák J, Hájek M. Synergistic application of antibiotics and hyperbaric oxygen therapy. Med Gas Res. 2026;16(2):176-177.*

References

1. Chmelař D, Rozložník M, Hájek M, Pospíšilová N, Kuzma J. Effect of hyperbaric oxygen on the growth and susceptibility of facultatively anaerobic bacteria and bacteria with oxidative metabolism to selected antibiotics. *Folia Microbiol (Praha)*. 2024;69:101-108.
2. Marzella L, Vezzani G. Effect of hyperbaric oxygen on activity of antibacterial agents. In: Oriani G, Marroni A, Wattel F, eds. *Handbook on hyperbaric medicine*. Milano: Springer Milan. 1996:699-713.
3. Hind J, Attwell RW. The effect of antibiotics on bacteria under hyperbaric conditions. *J Antimicrob Chemother*. 1996;37:253-263.
4. Bornside GH. Enhancement of antibiotic activity against Staphylococcus aureus by exposure to hyperbaric oxygen. *Appl Microbiol*. 1967;15:1020-1024.
5. Gade PAV, Olsen TB, Jensen PØ, et al. Modelling of ciprofloxacin killing enhanced by hyperbaric oxygen treatment in Pseudomonas aeruginosa PAO1 biofilms. *PLoS One*. 2018;13:e0198909.
6. Kolpen M, Appeldorff CF, Brandt S, et al. Increased bactericidal activity of colistin on Pseudomonas aeruginosa biofilms in anaerobic conditions. *Pathog Dis*. 2016;74:ftv086.
7. Kolpen M, Lerche CJ, Kragh KN, et al. Hyperbaric oxygen sensitizes anoxic pseudomonas aeruginosa biofilm to ciprofloxacin. *Antimicrob Agents Chemother*. 2017;61:e01024-17.
8. Lerche CJ, Schwartz F, Pries-Heje MM, et al. Potential advances of adjunctive hyperbaric oxygen therapy in infective endocarditis. *Front Cell Infect Microbiol*. 2022;12:805964.
9. Imlay JA. Cellular defenses against superoxide and hydrogen peroxide. *Annu Rev Biochem*. 2008;77:755-776.
10. Fang FC. Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. *Nat Rev Microbiol*. 2004;2:820-832.
11. Zheng M, Aslund F, Storz G. Activation of the OxyR transcription factor by reversible disulfide bond formation. *Science*. 1998;279:1718-1721.
12. Dwyer DJ, Belenky PA, Yang JH, et al. Antibiotics induce redox-related physiological alterations as part of their lethality. *Proc Natl Acad Sci U S A*. 2014;111:E2100-2109.
13. Winterbourn CC, Hampton, MB. Thiol chemistry and specificity in redox signaling. *Free Radic Biol Med*. 2008;45:549-561.
14. Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol Mol Biol Rev*. 2003;67:593-656.
15. Unden G, Bongaerts J. Alternative respiratory pathways of Escherichia coli: energetic efficiency, and regulation. *FEMS Microbiol Rev*. 1997; 21:143-157.
16. Huang C, Zhong Y, Yue C, He B, Li Y, Li J. The effect of hyperbaric oxygen therapy on the clinical outcomes of necrotizing soft tissue infections: a systematic review and meta-analysis. *World J Emerg Surg*. 2023;18:23.