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Improving antibiotic treatment of bacterial biofilm by hyperbaric oxygen therapy: Not just hot air

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ABSTRACT

Bacteria and fungi show substantial increased recalcitrance when growing as infectious biofilms. Chronic infections caused by biofilm growing microorganisms is considered a major problem of modern medicine. New strategies are needed to improve antibiotic treatment of biofilms. We have improved antibiotic treatment of bacterial biofilms by reviving the dormant bacteria and thereby make them susceptible to antibiotics by means of reoxygenation. Here we review the rationale for associating lack of oxygen with low susceptibility in infectious biofilm, and how hyperbaric oxygen therapy may result in reoxygenation leading to enhanced bactericidal activity of antibiotics. We address issues of feasibility and potential adverse effects regarding patient safety and development of resistance. Finally, we propose means for supplying reoxygenation to antibiotic treatment of infectious biofilm with the potential to benefit large groups of patients.

Introduction

In 2015, WHO initiated a "global action plan" to combat antimicrobial resistance, which is an increasing problem regarded as a major health challenge [1]. We aim to resolve the part of this problem which is related to the recalcitrance of chronic bacterial biofilm infections. By controlling the physiology of the bacteria, susceptible phenotypes may be induced, allowing antibiotics to kill the bacteria before development of resistance emerges. A possible role of the bacterial physiology, involving anaerobic metabolism with low levels of activity [2], for the failure of eradication of infectious biofilm by antibiotic treatment has become evident in cystic fibrosis (CF) patients with chronic lung infection with *Pseudomonas aeruginosa* biofilm, which is the most extensively studied type of biofilm infection [3].

Biofilm in chronic lung infection

Chronic bacterial pneumonia is the most serious complication in patients with CF. Despite frequent and intense antibiotic treatments using antibiotics with *in vitro* activity against the infecting bacteria, the bacteria which cause the infection to persist [3]. Even though the chronic lung infections of CF patients are caused by bacteria which often remain sensitive to antibiotics according to conventional susceptibility testing, it has not been clear why they are not eradicated by aggressive appropriate antibiotic treatment [4–6]. It can neither be explained as a development of conventional resistance mechanisms associated with changes in the genes of the bacteria nor due to insufficient penetration of the antibiotics into the lungs or the biofilms [7–9].

The chronic pneumonia in CF is dominated by bacteria such as *P. aeruginosa*, which survive in the patients' endobronchial mucus, where *P. aeruginosa* is aggregated in biofilm surrounded by numerous polymorphonuclear leukocytes (PMNs) [10]. Although the activity of PMNs is intense, the organization of *P. aeruginosa* in biofilm protects against the PMNs [11,12]. The contribution of biofilm formation to the tolerance against antibiotics in CF lungs is, however, less obvious. Even susceptibility testing of clinical isolates grown as biofilm has so far failed to recommend antibiotics that could improve the outcome of antibiotic treatment of CF patients with *P. aeruginosa* lung infection [13–15].

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Impaired availability of oxygen in biofilms in infected lungs

A focus area for us is the association between antibiotic tolerance and the chemical microenvironment since the presence of *P. aeruginosa* biofilm induces a host response which strongly affects the microenvironment in the infected endobronchial mucus [2]. This is due to the following: *P. aeruginosa* in biofilm strongly activate PMNs leading to intense consumption of molecular oxygen (O_2) [16] Such intense removal of O_2 is evident from the O_2-depleted endobronchial mucus and the large anoxic zones in fresh sputum from chronically infected CF patients [17–19]. The impaired tolerance to antibiotics of anoxic biofilm has long been recognized and connected to low metabolic activity [20,21].

The rapid consumption of O_2 by PMNs results in the conversion of O_2 to reactive oxygen species (ROS) during the respiratory burst [22] and in production of reactive nitrogen species by the inducible nitric oxide synthethase [23], which is activated in PMNs by bacteria and migration from the blood to the focus of infection [24,25]. O_2-depletion restricts *P. aeruginosa* to employ anaerobic metabolism, as evidenced by excretion of nitrous oxide (N_2O) in anoxic zones in expectorated CF mucus [18] and by upregulated genes for anaerobic metabolism in *P. aeruginosa* isolates from CF patients [26,27]. N_2O secretion is a characteristic signature of bacterial anaerobic respiration known as denitrification [28]. The poor yield of adenosine triphosphate (ATP) by denitrification [29] and the low amount of nitrate (NO_3^-) and nitrite in CF lungs [18] suggests slow anaerobic growth by *P. aeruginosa*. In this context, it is interesting that we have shown, that *P. aeruginosa* has very low growth rates in CF lungs [30], and this slow growth can be reproduced in O_2-free *P. aeruginosa* cultures supplemented with physiological amounts of NO_3^- [31]. Other biofilm-forming pathogens also exhibit slow growth in the hypoxic CF mucus [32,33] and slow growth may have strong influence on the outcome of antibiotic treatment, as slow bacterial growth is associated with increased tolerance to several antibiotics [34,35]. The slow growth indicates low metabolic activity due to the low energy yield by anaerobic metabolism. Low metabolic activity may cause reduced uptake of antibiotics and down-regulation of antibiotic targets [36]. In addition, O_2-depletion may inhibit the effect of antibiotics depending on bacterial production of lethal amounts of ROS by reducing ROS formation [37,38] as well as by attenuating the aerobic bacterial respiratory chain, impairing antibiotic efficacy [39]. Part of the impact of O_2 on the susceptibility to antibiotics may result from aerobic bacterial respiration stimulating the tricarboxylic acid (TCA) cycle, which is associated with improved effect of antibiotic treatment [40]. Accordingly, O_2-depletion may further increase bacterial tolerance to antibiotics by promoting anaerobic glycolysis instead of the TCA cycle. Moreover, several enzymes of the TCA cycle are activated by O_2 [41].

Increasing the susceptibility of bacteria in biofilms by hyperbaric oxygen treatment

We have shown in an agarose *P. aeruginosa* biofilm model that slow growing bacterial subpopulations in O_2-free zones can be made susceptible to antibiotics by reoxygenation [42–44]. Reoxygenation was established with hyperbaric oxygen treatment (HBOT), where biofilm was exposed to an atmosphere of 100% O_2 at 2.8 bar for 90 min. In combination with tobramycin treatment we saw that HBOT could enhance the killing of clinical *P. aeruginosa* isolates from CF patients grown as biofilm more than 100,000 times [44] and the killing of PAO1 biofilm more than 100 times in combination with ciprofloxacin [42,43]. HBOT also reduced the amount of tobramycin needed to achieve the clinically relevant biofilm bactericidal concentration (BBC) by more than 50% [44].

When the *in vivo* situation, with intense O_2-depletion caused by PMNs, was reproduced by growing *P. aeruginosa* biofilms anaerobically in agarose, HBOT generated oxygenated zones. These oxygenated zones contained aerobically respiring subpopulations with increased growth and susceptibility to ciprofloxacin, partly due to formation of bactericidal ROS [43] as found in biofilms grown at atmospheric O_2 [45]. Similarly, the enhanced susceptibility to tobramycin caused by HBOT was associated with reoxygenation of anoxic zones, with aerobic respiration and increased growth in *P. aeruginosa* biofilm [44]. However, evidence to substantiate the contribution of ROS formation to the reinforcement of the susceptibility to tobramycin by HBOT remains to be obtained. Likewise, influence of HBOT on antibiotic uptake, the TCA cycle and target expression await further examinations (Fig. 1).

The ability of HBOT to increase the killing of *Staphylococcus aureus* biofilm by tobramycin in an animal model of endocarditis [46] also emphasizes the potential of HBOT to increase the susceptibility to antibiotics in biofilms of other bacteria than *P. aeruginosa*.

Potential clinical significance of HBOT

It is highly relevant to know the effect of HBOT on the level of reoxygenation in biofilm infections to evaluate the possibility of enhancing the outcome of antibiotic treatment. As such knowledge may be difficult to achieve, we have estimated rates of O_2 consumption and diffusion to develop models that may predict the effect of applied O_2 on reoxygenation of infectious biofilm [47]. When considering that the air in the airway closely resembles the inhaled air [48] these models predict that reoxygenation by HBOT expands the oxygenated zones, where sensitivity to antibiotics is restored by more than 3 times in CF patients with chronic lung infection. The potential for using HBOT for treatment of patients with chronic bacterial lung infection is however limited by the risk of barotrauma in damaged lungs due to potential accumulation of trapped air. Trapped air is a risk factor for imposing acute, severe tissue lesions due to expansion of the trapped air in the decompression phase of HBOT. Accordingly, pneumothorax is an absolute contraindication [49]. Another challenge is the timing of HBOT with antibiotic administration, as HBOT should be applied during the period where the concentration of antibiotics reaches its maximum in the lung to achieve the best result, and patients are only allowed limited time daily with HBOT.

HBOT has clinically been used to improve antibiotic treatment of biofilm-related infections.

Such as brain abscesses [50,51], device-related infections [52] and chronic and refractory osteomyelitis [53].

However, more solid clinical evidence is needed to evaluate the feasibility of applying HBOT during antibiotic treatment of patients. On the other hand, the potential of adjuvant HBOT during biofilm-related *in vivo* infections has recently been highlighted in experimental studies by the increased bacterial clearing and a better outcome in rats with

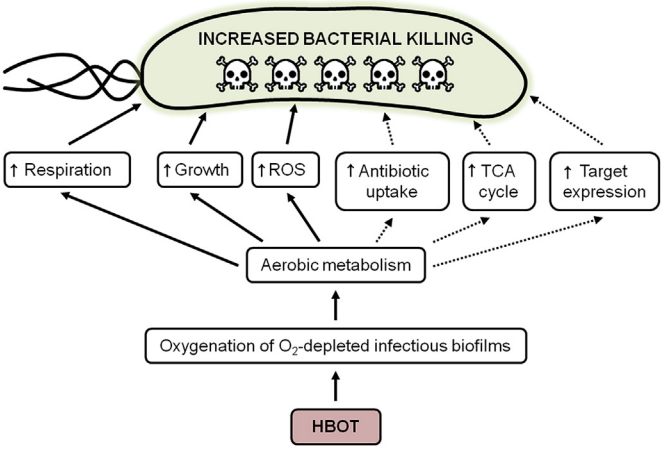

Fig. 1. Confirmed (solid lines) and putative (dotted lines) mechanisms leading to increased bacterial killing when applying HBOT during antibiotic treatment of infectious bacterial biofilms.

endocarditis caused by *S. aureus* when receiving adjuvant HBOT during treatment with tobramycin and linezolid [46,54]. A contribution of the host response to the improved outcome of adjuvant HBOT of biofilm infections *in vivo* should also be considered. In particular, HBOT may enhance the killing of *S. aureus* by PMNs [55].

Although the decreased BBC of tobramycin caused by HBOT indicates that HBOT enables the use of shorter treatment and thereby less antibiotics in line with WHO's recommendations [1] to prevent antimicrobial resistance (AMR), it is not known how adjuvant HBOT affects development of AMR. Lack of O_2 strongly reduces the induction of antibiotic resistance by sublethal amounts of antibiotic treatment [56], and it may be speculated that reoxygenation during adjuvant HBOT promotes the development of AMR if the lethal effect of the antibiotics is not achieved. However, the development of AMR during sublethal antibiotic treatment at atmospheric O_2 [56,57] has so far been achieved during overnight culturing or longer which is way beyond the typical sessions of 90 min with HBOT. Thus, further studies are needed to determine the influence of adjuvant HBOT on development of AMR.

Conclusion

In CF patients with chronic lung infections, antibiotic treatment is insufficient for eradication of bacteria regardless of resistance patterns. Supplemental HBOT has shown increased bactericidal effect of antibiotics, and cyclic treatment with HBOT combined with inhalation or intravenous administration of antibiotics can be a new option for the treatment. If our model proves clinically applicable, our findings from CF could be applied to much larger disease groups with biofilm infections where O_2-depletion may induce tolerant phenotypes (e.g. chronic obstructive pulmonary disease). It will potentially lead to better utilization of antibiotics, thereby reducing the total amount of antibiotics administered and improve the patient outcome.

Declaration of competing interest

No conflicts of interest.

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