International Journal of Antimicrobial Agents 50 (2017) 406–412

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Hyperbaric oxygen therapy augments tobramycin efficacy in experimental *Staphylococcus aureus* endocarditis

C.J. Lerche [a,*,1], L.J. Christophersen [a], M. Kolpen [a,b], P.R. Nielsen [c], H. Trøstrup [a], K. Thomsen [a], O. Hyldegaard [d], H. Bundgaard [e], P.Ø. Jensen [a,b], N. Høiby [a,b], C. Moser [a]

[a] Department of Clinical Microbiology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[b] Institute of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark
[c] Department of Pathology, Zealand University Hospital, Roskilde, Denmark
[d] Department of Anaesthesia, Centre of Head and Orthopedics, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[e] Department of Cardiology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 13 January 2017
Accepted 5 April 2017

Keywords:
Hyperbaric oxygen therapy
Host response
Biofilm
Hypoxia
Neutrophils
Oxidative stress

ABSTRACT

Staphylococcus aureus infective endocarditis (IE) is a serious disease with an in-hospital mortality of up to 40%. Improvements in the effects of antibiotics and host responses could potentially benefit outcomes. Hyperbaric oxygen therapy (HBOT) represents an adjunctive therapeutic option. In this study, the efficacy of HBOT in combination with tobramycin in *S. aureus* IE was evaluated. A rat model of *S. aureus* IE mimicking the bacterial load in humans was used. Infected rats treated subcutaneously with tobramycin were randomised into two groups: (i) HBOT twice daily ($n = 13$); or (ii) normobaric air breathing (non-HBOT) ($n = 17$). Quantitative bacteriology, cytokine expression, valve vegetation size and clinical status were assessed 4 days post-infection. Adjunctive HBOT reduced the bacterial load in the aortic valves, myocardium and spleen compared with the non-HBOT group ($P = 0.004$, <0.001 and 0.01, respectively) and improved the clinical score ($P < 0.0001$). Photoplanimetric analysis and weight of valve vegetations showed significantly reduced vegetations in the HBOT group ($P < 0.001$). Key pro-inflammatory cytokines [IL-1β, IL-6, keratinocyte-derived chemokine (KC) and vascular endothelial growth factor (VEGF)] were significantly reduced in valves from the HBOT group compared with the non-HBOT group. In conclusion, HBOT augmented tobramycin efficacy as assessed by several parameters. These findings suggest the potential use of adjunctive therapy in severe *S. aureus* IE.

1. Introduction

Staphylococcus aureus infective endocarditis (IE) is a serious acute infectious disease involving colonisation of the cardiac valves, endocardium or prosthetic material in the heart. The incidence of *S. aureus* IE is increasing and it has a reported mortality rate (1-year mortality) of up to 40%, which has not changed during the last 5 decades [1]. New therapeutic approaches, interventions and particularly optimisation of the initial treatment are required [2].

Staphylococcus aureus is a Gram-positive facultative aerobe, a versatile and ubiquitous pathogen that can infect any human tissue. *Staphylococcus aureus* causing endovascular infections are generally highly virulent and trigger a prompt inflammatory host response. To optimise treatment, expanded knowledge of the bacterial–host interplay in IE is needed. This complex interplay activates cascades of consecutive processes resulting in the recruitment of host defence cells and the release of multiple inflammatory cytokines. The pathogenesis of the host and pathogen interactions has been intensively studied for decades, mainly focusing on pathogen virulence factors [3,4], with less attention on the influence of the host response in relation to infection and treatment response.

The crucial key point in treating *S. aureus* IE, like any other severe infectious disease, is early [5,6] and sufficient high-dose antibiotic combination therapy [7] to retain infection control and minimise complications. Unfortunately, diagnostic delay and inadequate antibiotic treatment, especially in the initial phase of the IE course, are seen in a considerable portion of patients. A serious infection such as *S. aureus* IE involving endothelium damage, bacteraemia and septic dissemination to vital organs triggers an exaggerated host response, activating platelets and immune cells to the site of inflammation, which may lead to additional tissue and organ damage [8].

Polymorphonuclear leukocytes (PMNs) are the first line of defence against *S. aureus* [9]. Increased tissue oxygen consumption is a major

* Corresponding author. Department of Clinical Microbiology 93.01, Copenhagen University Hospital, Rigshospitalet, Juliane Maries Vej 22, 2100-DK Copenhagen, Denmark.
E-mail address: cjl@dadlnet.dk (C.J. Lerche).
[1] Present address: Department of Clinical Microbiology 93.01, Copenhagen University Hospital, Rigshospitalet, Juliane Maries Vej 22, 2100-DK Copenhagen, Denmark.

http://dx.doi.org/10.1016/j.ijantimicag.2017.04.025
0924-8579/

component of the response in severe sepsis [10], osteomyelitis [11], infected wounds [12] and biofilm infections [13] and is expected to be likewise for left-sided native valve *S. aureus* IE. Although PMNs are primarily dependent on glycolysis rather than oxidative metabolism for ATP generation, they require an oxygen supply to maintain the NADPH oxidase-driven respiratory burst, which generates reactive oxygen species required for pathogen killing. Several studies have shown that infected sites are severely depleted of oxygen, and hypoxia has been shown to inhibit the ability of PMNs to kill *S. aureus* [14,15].

Accumulating evidence has shown that several bactericidal antibiotics, such as β-lactams, fluoroquinolones and aminoglycosides, partly depend on bacterial aerobic respiration in addition to their target-specific killing mechanisms [16,17]. Therefore, the efficacy of these antibiotics is to some extent dependent on the availability of O_2 and the metabolic status of the bacteria [17,18]. The purpose of hyperbaric oxygen therapy (HBOT) as adjunctive treatment is to stimulate the aerobic respiration of pathogens and to re-oxygenate the infected and O_2-depleted tissue, thereby increasing pathogen susceptibility. In addition, HBOT may increase the capacity of the PMN respiratory burst against *S. aureus* as well as impairing exotoxin production, the latter being O_2 sensitive and can be inhibited at tissue partial pressures achievable with HBOT [11,19].

Therefore, we hypothesised that HBOT may augment the efficacy of the aminoglycoside tobramycin in the treatment of *S. aureus* experimental IE, especially in the early course of IE. HBOT as adjunctive therapy in the early course of IE may be of beneficial value for some of the abovementioned reasons. HBOT has already been proven to be beneficial in a variety of infectious diseases [20], mostly in deep-seated and recalcitrant infections such as necrotizing fasciitis [21], osteomyelitis [22] and chronic wounds [23]. Investigations of adjunctive HBOT in IE are scarce [24]. In a recent study [25], we showed poor efficacy of functional monotherapy with tobramycin in *S. aureus* experimental IE. The clinical consideration behind this study is that a substantial fraction of patients with methicillin-susceptible and methicillin-resistant *S. aureus* IE may be treated insufficiently (functional monotherapy or single-agent therapy) in the early phase of the disease.

The objective of this study was to evaluate the potential effects of HBOT in *S. aureus* IE. Therefore, a suboptimal antibiotic treatment was intentionally used in order to measure any potential effects of adjunctive HBOT in the experimental *S. aureus* IE model.

2. Materials and methods

2.1. Staphylococcus aureus strain

A penicillin-susceptible, methicillin-susceptible *S. aureus* strain (NCTC 8325-4) was used in this study. An inoculum was made from an overnight culture at 37 °C, was resuspended in fresh Luria–Bertani medium and was grown to log phase (optical density at 600 nm = 0.5). The inoculum was then centrifuged at 5000 rpm at 5 °C, was washed in saline (0.9%) and was diluted to the desired inoculum size of 0.5×10^7 CFU as described previously [25].

2.2. Experimental endocarditis in rats

All experiments were approved by The National Authority, and rats were maintained and handled in accordance with guidelines for animal research. High-grade aortic valve IE was established in rats as described previously [25]. In brief, male Wistar rats (225–250 g body weight) were anaesthetised with a mixture of Hypnorm (fentanyl 0.315 mg/mL and fluanisone 10 mg/mL), sterile water and midazolam (5 mg/mL) in 1:2:1 dilution. The tip of a sterile polyethylene catheter (Portex Ltd., Hythe, UK) was surgically placed at the aortic valve in the left ventricle of each animal via the right carotid artery and was verified by pulsation. Catheters were kept in situ for 24 h in order to damage the valve and were then surgically removed under general anaesthesia as outlined above. Subsequently, rats were injected intravenously with a bacterial suspension of 0.5×10^7 CFU of the *S. aureus* inoculum, inducing left-sided high-grade aortic valve IE.

2.3. Study design

The effect of HBOT has been shown to be dose-dependent [10,26]. In extensive pilot studies, we have found that HBOT given once daily in combination with tobramycin is insufficient to reduce the bacterial load significantly in some rats in a high-grade IE model (data not shown). For this reason, HBOT was intensified to twice daily (b.i.d.). Infected rats were randomised into two groups: one group receiving HBOT b.i.d. for 90 min with an 8-h interval in combination with tobramycin ($n = 13$); and another group receiving tobramycin under normobaric oxygen breathing (non-HBOT) ($n = 17$). Six catheter-inflicted non-infected sham control rats were included, including three receiving HBOT and three kept at normobaric conditions. A control arm of untreated rats was not included in this study but has been performed in previous work [25]. All animals were evaluated at 4 days post-infection (DPI) (Fig. 1).

2.4. Tobramycin treatment in the experimental model of infective endocarditis

Rats were treated with 20 mg/kg/day tobramycin (Nebcina®; Eurocept International B.V., Ankeveen, The Netherlands) subcutaneously initiated 1 DPI as described previously [25].

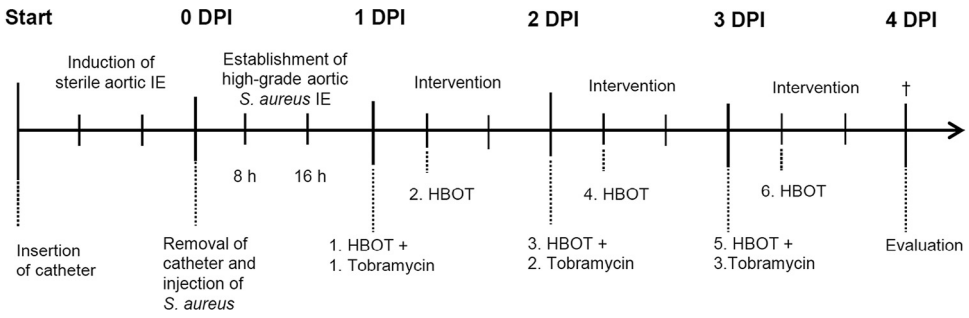

Fig. 1. Schematic timeline representation of experimental rat model of high-grade *Staphylococcus aureus* infective endocarditis (IE) receiving adjunctive hyperbaric oxygen therapy (HBOT) with 100% O_2 at 280 kPa (2.8 bar) for 90 min in combination with tobramycin (20 mg/kg/day subcutaneously). DPI, days post-infection. † sacrificed.