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TREATMENT AND PROPHYLAXIS - SHORT COMMUNICATION

High oxygen concentrations inhibit *Acanthamoeba* spp.

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Abstract

Efficacious treatments against *Acanthamoeba* Keratitis (AK) is challenging, often ineffective and linked to the intragenotype variation in the drug efficacy. Increased oxygen can facilitate host response and can inhibit some organisms. Herein, we report the effect of increased oxygen concentrations on *Acanthamoeba* spp. growth and its effect on ROS (reactive oxygen species) production. The exposition to pure oxygen could reduce cell growth by at least 60% for *Acanthamoeba castellanii* Neff, *Acanthamoeba polyphaga*, and *Acanthamoeba griffini*. The increase in ROS production confirming that oxygen cell's growth inhibition was due to oxidative stress. Further studies are needed to determine oxygen saturation level, time of oxygen exposition, and number of sessions needed to eliminate the parasite.

Keywords *Acanthamoeba* spp · Viability · Reactive oxygen species · Oxygen therapy

Introduction

Free-living amoebae (FLA) are eukaryotic, ubiquitous protozoa found in natural habitats such as soil and water. These organisms act as pathogenic parasites or by carrying pathogens micro-organisms such as bacteria or virus (Javanmard et al. 2017; Sente et al. 2016). Up to now, six genera have been reported as pathogens and opportunistic *Acanthamoeba* spp, *Naegleria fowleri*, *Balamuthia mandrillaris*, *Vahlkampfia*, *Vermamoeba*, and *Sappinia* (Javanmard et al. 2017). *Acanthamoeba*, as the most abundant genus of the FLA group, has been isolated from different habitats such as soil, water, air, dust, drinking water, sea water, contact lense solution, and recreational water (home aquaria, swimming pools) (Reyes-Batlle et al. 2014, 2016; Vijayakumar 2018). Until present, 22 genotypes have been reported from T1 to T22 (Motavalli et al. 2018). Several genotypes, namely T4, T2, T3, T5, T6, T11, T13, and T15 were reported as causative agents of *Acanthamoeba* keratitis (AK) and amoebic encephalitis (Castro-Artavia et al. 2017; Grun et al. 2014). The current therapy against *Acanthamoeba* presents several shortcomings, namely drug resistance (Seal 2003). Even

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thought, the development of new agents or strategies with amoebicidal effect remains a challenge for the scientific and medical communities. Recently, numerous studies have reported novel therapy approaches to treat *Acanthamoeba*'s infections including photodynamic hyperbaric and nanotechnology approaches (Hendiger et al. 2020; P. Maritschnegg et al. 2011; Siddiqui and A Khan 2020; Vázquez-Ortega et al. 2020).

Increased oxygen concentrations may enhance neutrophil function since studies have demonstrated as supplemental oxygen administration lowers the risk for post-operative infections (Greif et al. 2000; Hopf and Holm 2008) and inhibit some organisms (Mader et al. 1980). In the case of keratitis, there could be a topical effect of oxygen, as the cornea is exposed to the ambient gas, as well as improved host response with supplemental oxygen inhalation.

In the present study, the effect of high oxygen concentrations was evaluated against *Acanthamoeba* spp. growth and reactive oxygen species production, in vitro.

Material and methods

The effect of high oxygen concentrations on the trophozoite stage of *Acanthamoeba* was conducted using the alamarBlue™ method (Martín-Navarro et al. 2008; Sifaoui, et al. 2019; 2017; 2018) on four *Acanthamoeba* strains: *Acanthamoeba castellanii* Neff, genotype T4 (ATCC 30,010) type strain from the American Type Culture Collection; *Acanthamoeba griffini*, genotype T3 obtained in previous studies (González-Robles et al. 2014); *Acanthamoeba polyphaga*, genotype T4 (ATCC 30,461); and *Acanthamoeba quina,* genotype T4 (ATCC 50,241). Those strains were grown axenically in PYG medium (0.75% (w/v) proteose peptone, 0.75% (w/v) yeast extract, and 1.5% (w/v) glucose) containing 40 µg gentamicin ml^{-1} (Biochrom AG, Cultek, Granollers, Barcelona, Spain).

Trophocidal activity was evaluated as previously described (Martín-Navarro et al. 2008; Sifaoui et al. 2017; 2018). Briefly, *Acanthamoeba* strains were seeded in triplicate on a 96-well microtiter plate with 100 µL from a stock solution of 5×10^4 cells mL^{-1}. Finally, the alamarBlue™ Cell Viability Reagent (Bioresource, Europe, Nivelles, Belgium) was added into each well at an amount equal to 10% of the medium volume. The plates were then incubated for 96 h at 28 °C in a hermetic desiccant chamber.

This experiment was conducted at an altitude of 543 m with barometric pressure of 1025 hPa. The temperature was controlled at 22 °C. Using a vacuum, the total air inside the experimental chamber was replaced with pure oxygen. In order to ensure the saturation of oxygen, the chamber was kept under a continuous oxygen supply during the experiment. After 96 h, the emitted fluorescence was measured with an EnSpire microplate reader (PerkinElmer, Massachusetts, USA) at 570/585 nm. To highlight the effect of pure oxygen on *Acanthamoeba* spp., a statistical comparison was conducted using one-way analysis of variance (ANOVA). All analyses and graphics were done by GraphPad Prism version 8.0. Statistical significance was set at $p < 0.05$. The generation of intracellular reactive oxygen species (ROS) was detected using the CellROX® Deep Red fluorescent probe (Invitrogen, Madrid, Spain) as previously described (Cartuche et al. 2019; Sifaoui et al. 2019).

Results and discussion

The corresponding in vitro trophocidal activity results are summarized in Fig. 1. The pure oxygen exposure reduced cell growth by 80% for *A. polyphaga* and *A. griffini*. The one-way analysis of variance (ANOVA) illustrated that the biological activity was affected by the type of strains used with a $p < 0.0001$. *A. quina* was the most resistance strain to oxygen exposure, with a growth inhibition of 40%. The analysis of the production of ROS was processed using the CellROX Deep Red™ dye (Sifaoui 2019). Figure 2 shows that exposure of *Acanthamoeba* spp. with pure oxygen noticeably enhanced the red fluorescence reflecting the increase of ROS intracellular production. Recently, serval studies have confirmed the cell growth inhibition effect of hyperoxia in bacteria, fungi, and parasites (Kawamura et al. 2015; Memar et al. 2019), probably induced by oxidative stress (Koru et al. 2012). Oxidative stress is a phenomenon

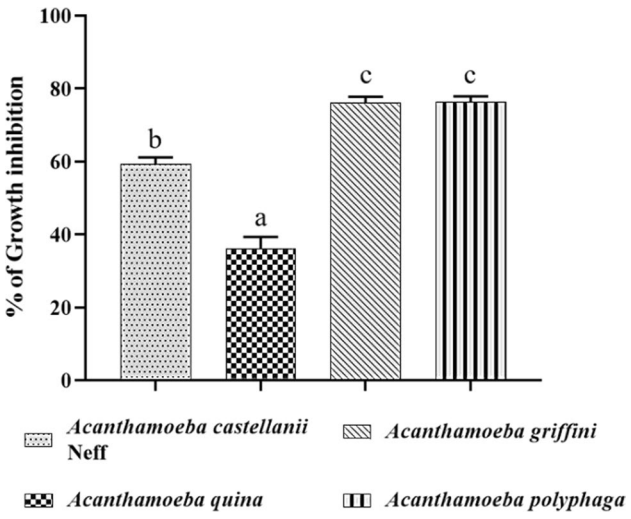

Fig. 1 Effect of the pure Oxygen on *Acanthamoeba* spp growth after 96 h. Results are representing in percentage relative to the negative control. Differences between the values were assessed using one-way analysis of variance (ANOVA). Data are presented as means ± SD ($N = 3$) and letters a–c reflect that means within strains with different letters are significantly different ($p < 0.05$)

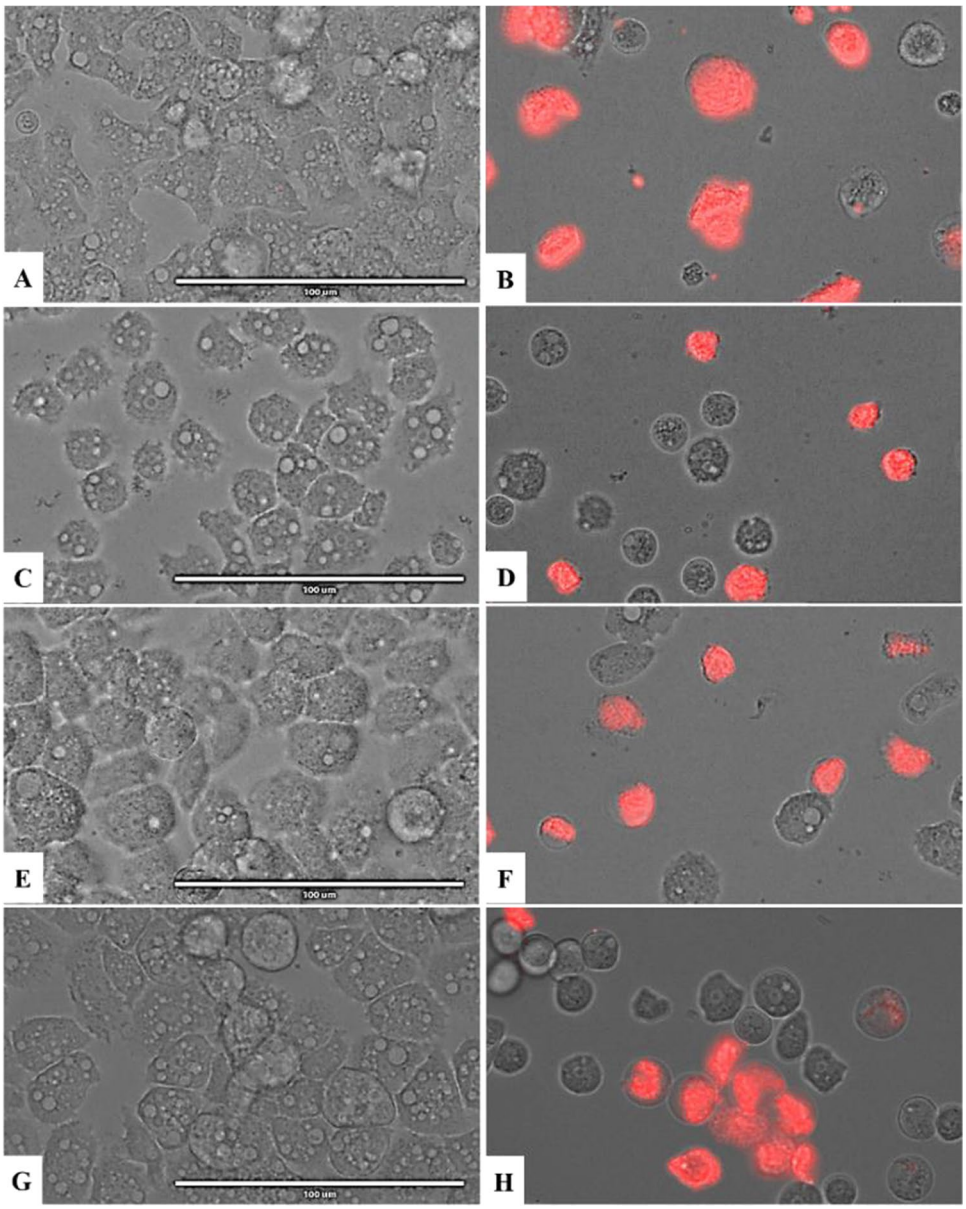

Fig. 2 Effect of effect of pure oxygen on ROS production of *Acanthamoeba castellanii* Neff (**B**), *Acanthamoeba quina* (**D**), *Acanthamoeba griffini* (**F**), and *Acanthamoeba polyphaga* (H) compared to the negative control *Acanthamoeba castellanii* Neff (**A**), *Acanthamoeba quina* (**C**), *A. griffini* (**E**), and *A. polyphaga* (**G**) after 96 h incubation. Images (×40) are representative of the cell population observed in the performed experiments. Images were obtained using an EVOS FL Cell Imaging System AMF4300, Life Technologies, USA

caused by an imbalance between ROS production and antioxidant production, which results in molecular and cellular damage (Tan et al. 2018). In *Acanthamoeba*, Motavalli et al. (2018) have demonstrated that an acute oxidative stress can damage the lipid bilayer and protein resulting in parasitic death (Motavalli et al. 2018). Several authors, have suggested that ROS causes oxidative stress and ultimately cell death in *Acanthamoeba* (Hajaji et al. 2017; Jha et al. 2015; Sifaoui et al. 2019).

This study demonstrates that *Acanthamoeba* spp. killing rates were significantly higher with exposure to pure oxygen for 96 h compared to air while at atmospheric pressure. Pure oxygen reduced by 70% the growth of *A. griffini* and *A. polyphaga*. The cell damage was likely mediated by an overproduction of ROS. We do not know if the effect on *Acanthamoeba* spp. if shorter durations than 96 h or if lower concentrations or if lower concentrations of oxygen were used. Hyperbaric oxygen therapy is widely used and has been demonstrated to be nontoxic in short-time applications (Wingelaar et al. 2019), and hence the development of this study. Nerveless, future experimental studies could determine a dose–response to eliminate both trophozoite and cyst stages. Indeed, combination with other amoebicidal therapies including photochemotherapeutic could be a great option. Animal studies with *Acanthamoeba* keratitis treated with high oxygen concentrations, which should include hyperbaric oxygen should be done. The information from animal studies could then be applied to humans infected by this organism in combination with the amoebicidal drugs. In vivo, studies in animals exposing

the eye to 100% oxygen and/or oxygen inhalation should be considered for the future.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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