ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2001, p. 3601–3602 Vol. 45, No. 12
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.12.3601–3602.2001


# Hyperbaric Oxygen as an Adjunct in Zygomycosis: Randomized Controlled Trial in a Murine Model

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Received 21 August 2000/Returned for modification 24 February 2001/Accepted 30 August 2001

**Zygomycosis was induced by injecting CD-1 mice with 5 mg of intraperitoneal deferoxamine and then $10^6$ CFU of intravenous and intrasinus *Rhizopus arrhizus*. The addition of hyperbaric oxygen (2.0 atm absolute twice daily) to amphotericin B did not improve survival over that achieved with amphotericin B and placebo air treatments.**

Zygomycoses are opportunistic fungal infections caused by ubiquitous organisms in the class Zygomycetes. (Some authors still prefer to use the term mucormycosis to describe mycoses caused by members of the Mucorales.) Zygomycosis is often associated with conditions such as diabetes (especially diabetic ketoacidosis), leukemia or lymphoma, immunosuppression following organ transplantation, severe burns, chronic steroid use, chemotherapy, and deferoxamine administration. Despite treatment with amphotericin B (AMB) and wide surgical debridement, mortality ranges from 23 to 100%, depending on the underlying condition (14). After life-saving surgical procedures, such as orbital exenteration and debridement of the face, skull base, and sinuses, survivors are often left disfigured.

An adjunctive therapy that could improve the morbidity and mortality of this disease is needed. Hyperbaric oxygen (HBO) has been suggested as a potential adjunct based on the following pathophysiological processes. Zygomycosis involves fungal invasion of the vasculature with embolism, thrombosis, tissue hypoxia, and lactic acidosis. Hypoxia impairs normal wound healing and infection-fighting processes and can inhibit the oxidative mechanism of AMB in vitro (12). Lactic acidosis enhances fungal growth, creating a vicious circle. HBO can relieve tissue hypoxia, restore oxygen necessary for the granulocyte respiratory burst, restore normal fibroblast function, relieve tissue lactic acidosis (9), and provide oxygen for the oxidative mechanisms of AMB. In addition, in vitro studies have shown that high partial pressures of oxygen are fungistatic or fungicidal and are additive with AMB (8). A retrospective review (14) of 145 patients with rhino-orbital-cerebral mucormycosis showed that survival in patients with bilateral involvement was 22% (4 of 18) with standard therapy (AMB and/or surgery) compared to 83% (5 of 6) in patients with standard therapy plus HBO ($P = 0.0285$). Another retrospective study (7) reported that survival was 33% (2 of 6) in patients treated with standard therapy, compared to 66% (4 of 6) in patients treated with standard therapy plus HBO.

The purpose of this study was to determine if HBO at 2.0 atm absolute (ATA) (200% surface equivalent) for 2 h twice a day (BID) has an additive effect on survival over AMB alone in a deferoxamine-induced murine model of disseminated rhino-orbital-cerebral zygomycosis.

(This study was presented at the Undersea and Hyperbaric Medical Society Annual Meeting in Boston, Mass., 26 to 29 June 1999.)

Institutional Animal Care and Use Committee approval was obtained prior to the study. Methods were as follows. On day 1, female CD-1 mice (weighing 22 to 24 g) were given intraperitoneal (i.p.) injections of 5 mg of deferoxamine (1). After a 3-h delay, mice were anesthetized (10) and inoculated with $10^6$ CFU (6) of *Rhizopus arrhizus* (sensitive to AMB in vitro and in vivo [11]) via the tail vein and ethmoid sinus. Mice were randomized into the following groups: group 1 HBO (2.0 ATA for 2 h BID) and AMB (0.31 mg/kg of body weight i.p. once a day [QD]); group 2, air (0.21 ATA of oxygen for 2 h BID) and AMB (0.31 mg/kg i.p. QD); and group 3, no treatment. All mice received subcutaneous buprenorphine (Sigma), 1 mg/kg BID, for analgesia (10). Therapy began 12 h after inoculation (designated as day 2). Two separate cycles were conducted, using a total of 58 mice in the first and 88 mice in the second.

Two deaths occurred approximately 15 h after inoculation; 14 deaths occurred prior to day 3. Deaths continued until day 10. Survival on day 14 was as follows: group 1, 42% (21 of 50); group 2, 46% (22 of 48); group 3, 31% (15 of 48). Histopathologic analysis with Gomori's methenamine silver staining revealed branching hyphae in 100% (5 of 5) of brains of group 3 animals. An analysis of the complete survival experience (Wilcoxon rank sum test) revealed that treatment with either HBO-AMB ($P = 0.01$) or air-AMB ($P = 0.067$) improved survival over no treatment by reducing the initial death rate and increasing the survival rate. However, there was no significant difference between the HBO-AMB and air-AMB groups ($P = 0.72$).

A multicenter group of medical mycologists (11) com-

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mented that it is very difficult to achieve reproducible, fatal experimental challenges with molds that resemble the clinical situation. Models of murine zygomycosis have utilized streptozotocin-induced diabetic ketoacidosis (13), deferoxamine (1), and alloxan-induced diabetes with and without deferoxamine (2) to predispose animals to infection. However, none utilize the natural route of infection—inhalation. Instead of developing isolated rhino-orbital-cerebral infections, mice that received intrasinus inoculations developed disseminated infections (13).

Mortality in patients predisposed to zygomycosis by deferoxamine administration ranged from 89 to 100% (5, 14). Deferoxamine predisposes the host to infection with zygomycetes by acting as a siderophore, that is, by binding iron in a form that the fungus can use as a growth factor, thereby enhancing the virulence and pathogenicity of *R. arrhizus*. In addition, deferoxamine may alter granulocyte and lymphocyte functions by interfering with iron-catalyzed peroxidase production of free radicals that are important for killing fungi (1, 5, 14).

In this study, an intrasinus inoculation was initially attempted in mice pretreated with deferoxamine. However, mortality rates were very low; therefore, mice were inoculated via both intravenous and sinus routes. Because the objective of this study was to determine whether HBO had an additive effect, a low dose of AMB (0.31 mg/kg) (11) was utilized in order to attain approximately 50% survival in the air-AMB group. HBO is not currently the standard of care in mucormycosis. The optimal treatment pressure may be 2.5 to 3.0 ATA, which is utilized in noncerebral necrotizing infections (3). However, intracranial infection can lower seizure threshold. Therefore, HBO was administered at 2.0 ATA for 2 h BID, based on a mucormycosis clinical series (7). Mice were observed before, periodically during, and after HBO treatment. As part of the disease process, some mice exhibited tremors. However, none had any abnormal movements during HBO treatment that were not already present. Fourteen days was chosen as the end point, because the majority of deaths occurred within 2 weeks in a clinical series (4) and within 1 week in murine models (1, 2, 11, 13).

In conclusion, treatment with either HBO-AMB ($P = 0.01$) or air-AMB ($P = 0.067$) improved survival function over no treatment. However, the addition of HBO to AMB did not improve survival function over AMB and placebo air treatments ($P = 0.72$) in this murine model of zygomycosis. Based on pathophysiological and clinical data, HBO appears to be beneficial in zygomycosis. The HBO regimen utilized in this study was not harmful; however, it may have been too mild to impact survival. In future studies, more aggressive HBO treatment should be utilized (3).

We acknowledge the following individuals for their contributions: Ana Espinel-Ingroff (Department of Infectious Diseases, Medical College of Virginia) for providing *R. arrhizus*; Richard E. Tracy (Department of Pathology, LSUMC) for interpreting the histopathologic specimens; Bruno C. Jubelin (Section of Emergency Medicine, LSUMC) for performing the statistical analysis; Drew Sallean (College of Medicine, LSUMC) for conducting microbiologic techniques; and Macy Vidrine, Ray Braquet, Uri Gedalia, Michael Haneman, Katy Thorn, Tricia Thorn, John Wilson, and Gerald Wells for technical assistance.

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