Hyperbaric oxygen therapy and other adjunctive treatments for zygomycosis

A. Tragiannidis1,2 and A. H. Groll1
1) Second Department of Paediatrics, Aristotle University, AHEPA Hospital, Thessaloniki, Greece and 2) Infectious Disease Research Programme, Centre for Bone Marrow Transplantation and Department of Paediatric Haematology/Oncology, University Children’s Hospital, Münster, Germany

Abstract

Zygomycetes are increasingly reported as a cause of life-threatening invasive fungal infections in profoundly immunocompromised patients and in those with diabetic ketoacidosis. Zygomycosis, typically presents as soft tissue, rhino-orbitocerebral, pulmonary or disseminated disease and is characterized by rapid clinical progression and high mortality rates. Treatment with amphotericin B lipid formulations in combination with surgery and, perhaps, the addition of caspofungin offers the best chance for survival; posaconazole, a new antifungal triazole, is increasingly used for consolidation or maintenance therapy. Because of the poor prognosis of zygomycosis, particularly in immunocompromised cancer patients, adjunctive treatments such as hyperbaric oxygen therapy, use of immunomodulatory cytokines, and in vivo iron starvation continue to be explored. However, although each of these modalities is based on a plausible scientific rationale and has been helpful in the management of individual patients, there is no clinical evidence for their general effectiveness as adjunctive treatments in patients with zygomycosis. Further experimental and clinical investigations are necessary to determine whether and how these treatments can impact on outcome and to determine which patients and which types of infection may benefit from them.

Keywords: Cytokines, hyperbaric oxygen, iron chelation, zygomycosis

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Introduction

Over the past decade, zygomycosis has been increasingly recognized as an important invasive fungal infection, particularly in patients with haematological malignancies, haematopoietic stem cell transplantation (HSCT), poorly controlled diabetes mellitus, and those treated with the iron chelator, deferoxamine. The agents of zygomycosis (Rhizopus, Mucor, Rhizomucor, Cunninghamella and Absidia spp.) are a group of biologically diverse, primarily opportunistic and mostly airborne pathogens of wide ecological distribution. Infections in humans are characterized by aggressive growth across all anatomical barriers with angioinvasion, thrombosis, haemorrhagic infarction and extensive tissue necrosis [1–8].

The major clinical manifestations of zygomycosis include rhino-orbitocerebral, pulmonary and disseminated infection; primary gastrointestinal and primary cutaneous infections are less frequent [1]. Depending on the underlying condition, the site of infection and the initiation of antifungal therapy, zygomycosis is associated with high mortality rates in the range of 35–70%, and close to 100% in disseminated disease with cerebral involvement [9]. Current approaches to management include the prompt initiation of antifungal chemotherapy, surgical debridement as feasible, restoration of immunologic or metabolic deficiencies, stabilization of the infection during compromised host status, and treatment until the resolution of all signs and symptoms [10]. Although there is currently expert consensus that amphotericin B (AmB) lipid complex or liposomal amphotericin B (L-AmB) are first choices for initial treatment, the optimal doses and uses for targeting different body sites remain unclear [11]. An emerging, although non-approved, option for second-line, consolidation or maintenance treatment is posaconazole and, perhaps, the combination of AmB with caspofungin [12]. However, despite advances in both the understanding and
management of zygomycosis, outcome remains poor and adjunctive treatments continue to be explored.

In this article, we review the theoretical and experimental foundations and clinical data on hyperbaric oxygen treatment (HBOT), adjunctive use of cytokines, and iron depletion with newer chelators as adjunctive treatments for zygomycosis. Other adjunctive measures, such as intra-arterial, aerosolized and intralesional administration of antifungal agents, or irrigation of infected tissues with antiseptics or antifungals, are not considered.

**Hyperbaric Oxygen Treatment**

The use of hyperbaric oxygen as adjunctive treatment for zygomycosis has been reported since the 1970s [13–17]. Although HBOT has been recommended and used in an extremely wide variety of medical conditions, the Undersea and Hyperbaric Medical Society endorses its use only in a few indications for which there is thought to be scientific evidence or validated clinical experience and which are accepted by the US Centers for Medicare and Medicaid Services for reimbursement [18]. Pressurized hyperoxygenation (i.e. exposure to oxygen at high ambient pressures) greatly increases the oxygen transport capacity in blood and the oxygen pressure gradient across capillary membranes, thereby augmenting tissue oxygenation [19, 20]. This is believed to be beneficial in situations of hypoperfusion and anaerobism, and is supported by experimental data that have demonstrated direct antimicrobial activity, restoration or enhancement of cellular defences, synergistic effects with antimicrobials, and restoration or augmentation of tissue repair [21,22]. During HBOT, 100% oxygen is administered in a monoplace or multiplace chamber through masks, tightly fitting hoods or endotracheal tubes. Inside the chambers, pressure is usually increased to c. 250–280 kPa, equivalent to a depth of 15–18 m of water and resulting in a PaO2 of approximately 1200–2000 mmHg. The recommended duration of a session (‘dive’) varies between 90–120 min with periodic air breaks to minimize the risk of oxygen toxicity; for longterm conditions, patients may receive 20–40 sessions administered according to a once-per-day to three-times-per-day schedule. The cost charged is USD 12 000–20 000 per 30 sessions [20].

Hyperbaric oxygen treatment is usually well tolerated and is associated with a low risk of adverse events. In general, if pressures exceed 300 kPa and the duration of treatment is more than 120 min, central nervous system (CNS), pulmonary, optic and middle ear symptoms may occur as a result of oxygen toxicity [19]. Overall, severe CNS symptoms may occur in 1–2% of patients, symptomatic reversible barotrauma in 15–20%, pulmonary symptoms in 15–20% and reversible blurred vision in up to 20% of patients. Untreated pneumothorax represents the only absolute contraindication for HBOT, whereas relative contraindications include a history of grand mal seizures, fever, congestion, emphysema, severe maxillofacial/head damage and pregnancy [19].

Zygomycoses are acute, angioinvasive, necrotizing tissue infections, that are characterized by invasion of blood vessels, thrombosis, infarction, tissue hypoxia and lactic acidosis [10]. HBOT has been shown to have direct antifungal activity in vitro at pressures <10 atmospheres (atm) absolute that is attributed to increased production of oxygen-based free radicals under hyperoxic conditions [17]; fungal mutants lacking antioxidant enzymes have an increased susceptibility to killing by HBOT that may be restored by the induction of antioxidant enzymes [23]. In addition, HBOT may have several indirect antimicrobial effects, including reversal of growth-promoting lactic acidosis, restoration of phagocytosis and augmentation of the oxidative burst by polymorphonuclear leukocytes (PMLs), and enhancement of the antifungal action of AmB [21,24]. Finally, HBOT contributes to tissue healing by increasing tissue oxygen levels, restoring normal fibroblast function, increasing collagen deposition, and promoting the secretion of inflammatory cytokines and angiogenesis [21,25,26].

In vivo, in deferoxamine-treated CD-1 mice with experimental disseminated zygomycosis, there was no added survival effect of HBOT as an adjunct to AmB, in comparison with AmB plus placebo air treatment [27]. However, the pressure used in these experiments (i.e. 2 atm absolute in two daily sessions) was comparatively low and applied without concomitant hyperoxygenation; moreover, an intravenous injection model may not be entirely representative of deep tissue infection as observed in humans; thus, the results of these experiments unfortunately remain inconclusive.

Data regarding the clinical efficacy of HBOT in invasive fungal infections are still limited. In an early retrospective study of 13 patients with rhinocerebral zygomycosis, six of whom received HBOT in addition to AmB and surgery, better survival in patients receiving HBOT was observed [28]. Segal et al. recently reported on 14 patients with zygomycosis or aspergillosis treated over a 12-year period with HBOT as an adjunct to antifungal chemotherapy and surgery. Seven of the patients survived and none experienced adverse events related to HBOT [29]. A contemporary literature review identified 28 cases in which zygomycosis was treated adjunctively with HBOT [30]. Underlying conditions included diabetes mellitus (61%), trauma (18%), haematological malignancies (11%), alcoholic liver disease (7%) and there was...
treatment with systemic corticosteroids (3%), no underlying disease (11%) of cases. The most commonly affected site of infection was sino-orbitocerebral in 75% of patients, followed by soft tissues (14%) and the lung (7%). Most of the patients received two sessions of HBOT per day for 90–120 min at a pressure of 2–3 atm absolute. In most patients, HBOT was administered postoperatively (23/25 with surgery, 92%). All but two patients also received antifungal treatment with AmB. The overall survival rate at end of treatment was 86%. Whereas survival was 94% in diabetes patients, it was poor (one of three) in patients with haematological malignancies or HSCT. There were no deaths among patients with trauma or without underlying disease. All patients who died had sino-orbitocerebral disease. Prolonged courses of hyperoxygenation (ten or more administrations) were associated with higher survival [30]. The impressively high survival rate of reported patients treated with adjunctive HBOT, however, may have been biased by several factors. Most patients in this series had correctable predisposing conditions; patients who did not respond to HBOT may be under-reported in the literature, and the improved survival rate among patients who received extended sessions may represent a survival bias as those who responded were more likely to continue hyperoxygenation treatment [30].

Thus, although there appears to be a scientific rationale for adjunctive HBOT in the treatment of zygomycosis, the clinical evidence is only anecdotal and does not allow for conclusions about its general clinical efficacy, nor about host- or disease-specific conditions of patients who might benefit from it.

**Adjunctive Cytokines**

Although the antifungal activity of PMLs and macrophages against Zygomycetes and the mechanisms involved in this activity were elucidated some time ago, few new data exist to enable better understanding of host defences against these organisms and the role of cytokines [31].

Polymorphonuclear leukocytes and macrophages are well known to constitute an important defensive mechanism against the agents of zygomycosis [32], providing a rationale for the use of granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferon-γ (IFN-γ) as adjunctive treatment beyond the setting of granulocytopenia. G-CSF and GM-CSF have been shown to increase phagocytosis, oxidative burst and fungicidal activity of PMLs [33–37], and IFN-γ to induce a T-helper cell type 1 (Th1) immunological response that favours resistance to invasive fungal infections and enhances PMLs’ antifungal activities [32,37,38].

Early experiments conducted to assess the ability of G-CSF to enhance PML activity against opportunistic fungal pathogens have demonstrated that G-CSF is able to enhance PML-mediated killing of *Rhizopus arrhizus* and induces a sustained respiratory burst in response to extracts of the organisms, implicating a possible therapeutic role for G-CSF as a response-modifying agent during infection [39]. A more recent series of experiments examined the comparative antifungal function of PMLs against hyphae of *Rhizopus oryzae*, *Rhizopus microsporus* and *Absidia corymbifera*, and evaluated the effects of IFN-γ GM-CSF, alone or combined, on PML antifungal function against these Zygomycetes [31]. In this study, human PMLs had a reduced capacity to mount an oxidative burst in response to both *Rhizopus* species and to induce hyphal damage of these Zygomycetes, in comparison with its response to *A. corymbifera*. However, IFN-γ and GM-CSF augmented PML-induced hyphal damage of all three Zygomycetes in a time-dependent manner. Furthermore, treatment of PMLs with the combination of cytokines enhanced the release of TNF-α in response to *R. microsporus* and *A. corymbifera*, but not in response to *R. oryzae* hyphae. By comparison, IFN-γ inhibited interleukin-8 (IL-8) release in response to hyphae of the three Zygomycetes. The results of these experiments suggest intergenus differences in host response to Zygomycetes and a potential role for IFN-γ and GM-CSF in the management of invasive zygomycosis.

Granulocyte CSF and GM-CSF have been used in a limited number of cases of zygomycosis as adjunctive treatment with favourable outcomes [40,41]. Although individual patients with extensive or refractory disease may benefit from the use of adjunctive cytokine treatment, further studies are needed to assess the general utility of IFN-γ, G-CSF or GM-CSF as adjuncts to antifungal chemotherapy.

**Iron Chelation Treatment**

Iron is required by virtually all microbial pathogens for growth and virulence. Patients with elevated levels of available serum iron are susceptible to infection by Zygomycetes, but not necessarily to infection by other fungi [42,43]. Iron metabolism plays an important role in infections with Zygomycetes and, paradoxically, an iron chelator, deferoxamine, has been found to promote zygomycosis. Deferoxamine, by its specific chemical structure, acts as an iron xenosiderophore for Zygomycetes, explaining the clinical observation of an association between zygomycosis and hypertransfusion [44]. This observation is well supported by animal models of experimental disseminated zygomycosis that uniformly
demonstrate the detrimental effects of deferoxamine on survival [44–47]. Indeed, a very comprehensive literature analysis of the epidemiology, presentation and outcome of zygomycosis revealed that zygomycosis occurring in the context of deferoxamine treatment was associated with the highest rate of disseminated infection compared with any other underlying condition [9].

By contrast, however, newer chelators such as deferoxamine, and deferasirox do not act as siderophores, but have been shown to have antifungal activity against Zygomycetes in vitro and in vivo [48,49]. In experimental investigations performed by Ibrahim et al., deferasirox had static activity in vitro against R. oryzae at 24 h, but was fungicidal at 48 h of incubation. In vivo, in diabetic ketoacidotic mice with disseminated R. oryzae infection, deferasirox was as effective as L-AmB, improving survival and decreasing brain fungal burden, and both drugs were more effective than placebo in non-iron-overloaded animals. Administration of free iron with deferoxamine reversed protection, confirming that the mechanism of protection was iron chelation [50]. In a subsequent, exceedingly well designed series of experiments, the authors demonstrated by molecular analysis effective iron chelation from R. oryzae by deferasirox and fungicidal activity in vitro against 28 of 29 clinical isolates of Mucorales at concentrations below clinically achievable serum levels. When administered to diabetic ketoacidotic or neutropenic mice with mucormycosis, deferasirox significantly improved survival and decreased tissue fungal burden, with an efficacy similar to that of L-AmB. Deferasirox treatment also enhanced the host inflammatory response to mucormycosis. Finally, deferasirox synergistically improved survival and reduced tissue fungal burden when combined with L-AmB.

These data collectively support the clinical investigation of iron starvation as adjutantive therapy to improve outcomes in cases of disseminated mucormycosis. To date, however, there are only anecdotal clinical data on the use of deferasirox in patients with invasive zygomycosis [51,52]. Although one case report describes an immediate and striking effect of the co-administration of deferasirox in a patient with rhino-cerebral mucormycosis [51]. Soummer et al. [52] reported progressive tissue invasion and treatment failure in the case of co-administered deferasirox in a patient with systemic intra-abdominal and intrathoracic zygomycosis. Whether treatment failure in the latter case reflected an already catastrophic situation at baseline, or was related to poor bioavailability or to lack of efficacy against the isolate, as hypothesized by the authors, remains unclear. Clearly, further studies are warranted to evaluate combined antifungal and iron chelation therapy in patients with invasive zygomycosis to assess the safety of this approach.

Conclusions

Because of the poor prognosis of zygomycosis, particularly in immunocompromised cancer patients, adjutantive treatments such as HBOT, use of immunomodulatory cytokines, and in vivo iron starvation continue to be explored. However, although each of these modalities has a plausible scientific rationale and has been helpful in the management of individual patients, there is no clinical evidence for their general effectiveness as adjutantive treatments in patients with zygomycosis. Further experimental and clinical investigations are necessary to determine whether and how these treatments can with which impact on outcome and to determine which patients, infections, may benefit from them.

Transparency Declaration

The authors declare no conflicts of interest.

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