Medical Mycology Case Reports 16 (2017) 16–19

Contents lists available at ScienceDirect

Medical Mycology Case Reports

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

Successful treatment of azole-resistant invasive aspergillosis in a bottlenose dolphin with high-dose posaconazole

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ARTICLE INFO

Keywords:
Aspergillus fumigatus
Azole resistance
Posaconazole
Bottlenose dolphin
Antifungal therapy

ABSTRACT

Invasive aspergillosis due to azole-resistant *Aspergillus fumigatus* is difficult to manage. We describe a case of azole-resistant invasive aspergillosis in a female bottlenose dolphin, who failed to respond to voriconazole and posaconazole therapy. As intravenous therapy was precluded, high dose posaconazole was initiated aimed at achieving trough levels exceeding 3 mg/l. Posaconazole serum levels of 3–9.5 mg/l were achieved without significant side-effects. Follow-up bronchoscopy and computed tomography showed complete resolution of the lesions.

1. Introduction

Azoles remain the corner-stone of prevention and treatment of aspergillus diseases, including acute invasive aspergillosis (IA) [1]. However, the clinical use of azoles is threatened by the emergence of azole resistance in *Aspergillus fumigatus*, the primary cause of IA in many regions of the world [2]. Resistance that arises through the environmental use of azole fungicides is believed to be an important driver of azole-resistant cases [3]. This route of resistance development proves a clinical challenge with cases of azole-resistant IA occurring in patients without previous azole therapy [4,5], and cases of azole-susceptible and azole-resistant *A. fumigatus* co-infection [6]. Resistance mutations commonly confer resistance to multiple azole drugs, including the recently clinically-licensed isavuconazole [7]. However, based on in vitro and experimental models it was hypothesized that low-level azole-resistant *A. fumigatus* infection might be successfully treated with voriconazole or posaconazole provided that the treatment dose and exposure could be increased [8].

We describe a case of azole-resistant IA in a bottlenose dolphin that was successfully managed by increasing posaconazole exposure.

2. Case

A 10 year old female captive bottlenose dolphin (*Tursiops truncatus*), weighing 175 kg, was treated with antibiotics for bacterial pneumonia caused by *Vibrio alginolyticus*. Treatment follow-up was conducted with blood samples, CT-scans, bronchoscopies, and protected brush samples from the tracheal and/or bronchial mucosa for histopathology and bacterial and fungal culture (Fig. 1).

The bacterial pneumonia required long-term antibiotic treatment and 6 months into therapy she developed multiple white, raised lesions in the trachea and bronchi, one of which was sampled with protected brush during bronchoscopy. No fungi were cultured but based on the bronchoscopic appearance of the lesions a *Candida* infection was suspected. Treatment with oral voriconazole with a loading dose of 5.5 mg/kg per day during 3 days and a maintenance dose of 5.5 mg/kg per week (given in one single dose) was started. Follow-up bronchoscopies were performed one, two and three months after voriconazole treatment was started, showing no improvement. Fungal culture of a protected brush sample, taken at one and two months of voriconazole therapy remained negative, but at three months *A. fumigatus* was cultured. Retrospective analysis of a serum sample indicated the presence of circulating galactomannan (GM; GM-index 6.5). The *A. fumigatus* isolate was sent to the Mycology Reference Laboratory and

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http://dx.doi.org/10.1016/j.mmcr.2017.03.005
Received 16 January 2017; Received in revised form 22 March 2017; Accepted 28 March 2017
Available online 01 April 2017

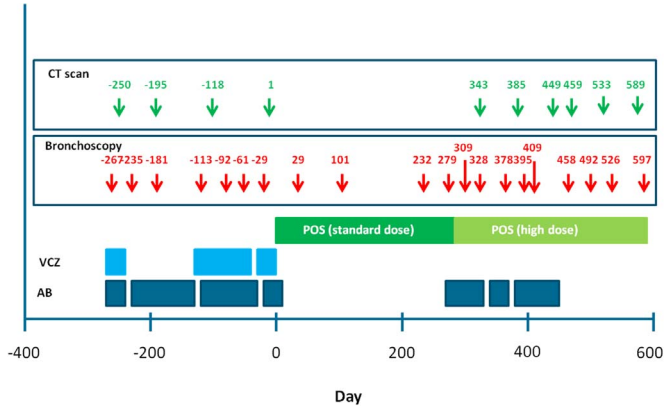

Fig. 1. Overview of bronchoscopy's and computed tomography's (CT) performed as part of the management of the respiratory infections in the bottlenose dolphin and the antimicrobial treatments. AB, antibacterial therapy; VCZ, voriconazole; POS, posaconazole. Day 0 represents the first day of posaconazole therapy.

was found to be resistant to voriconazole with a MIC of > 16 mg/l and a posaconazole MIC of 0.5 mg/l. The strain harbored the TR_{46}/Y121F/T289A resistance mutation in the cyp51A-gene. Treatment was then changed to posaconazole at a dose of 600 mg/day (day 0). The oral solution formulation of posaconazole was used, which was incorporated in gelatin capsules and placed into the fish she was fed. The daily dose was divided into two doses and administered in the morning and late afternoon. Posaconazole therapeutic drug monitoring was performed aiming at a target trough level of > 1 mg/l. The clinical condition of the dolphin initially improved and follow-up bronchoscopy on days 29, 101, and 232 showed stable disease. Monitoring of posaconazole plasma levels confirmed levels of > 1 mg/l. However, the clinical condition of the dolphin deteriorated and a new bronchoscopy on day 279 showed increased lesion size. The posaconazole dose was increased on day 286 to achieve plasma levels above 2 mg/l. However, this did not result in clinical improvement nor reduction of the size of the trachea lesions.

After 344 days of posaconazole therapy, a higher exposure target was advised based on preclinical studies, aiming at a plasma level of > 3 mg/l [8]. This drug level was first achieved on day 356 of posaconazole therapy. After initial clinical deterioration and stable size of the lesions, the dolphin started to improve clinically, which was supported by resolution of the lesions on the CT-scan (Fig. 2). The improvement further was confirmed with bronchoscopy showing complete resolution of the lesions on day 459. Posaconazole plasma levels varied between 3 and 9.5 mg/l, with doses ranging from 800 to 1800 mg/day (Fig. 3). After a total treatment duration of 593 days posaconazole therapy was discontinued.

The *A. fumigatus* isolate recovered from the dolphin was identified using beta-tubulin sequencing. MIC-testing using the EUCAST microdilution reference method showed a MIC of 1 mg/l for amphotericin B, high level-resistance (MIC > 16 mg/l) for itraconazole and voriconazole, and low-level resistance for posaconazole, 0.5 mg/l. A TR_{46}/Y121F/T289A resistance mutation was identified.

3. Discussion

We describe a case of pulmonary IA due to *A. fumigatus* in a bottlenose dolphin that was complicated by azole resistance. The fungus was cultured from plaques in the trachea and bronchi that were visualized through bronchoscopy, and computed tomography showed pulmonary lesions consistent with fungal disease. Furthermore, circulating GM was detected in the serum at diagnosis. Although GM detection is a well validated diagnostic tool in humans for the diagnosis of invasive aspergillosis and for treatment response evaluation [9,10], the performance of the assay in dolphins remains unclear. As IA is the most commonly reported in fungal disease in captive and free-ranging bottlenose dolphins, [11–13] further studies are needed to determine the performance of this biomarker in dolphins and other cetaceans. In addition to bronchoscopy, the presence of circulating GM would help to diagnose *Aspergillus* infection and possibly be useful for monitoring of treatment response. GM however does not identify the species of *Aspergillus* that is causing the fungal pneumonia, nor does it provide information regarding the presence of resistance. Additional tests would be required either through culture or molecular tests of respiratory specimens. PCR-tests that identify the most prevalent *Aspergillus* species as well as the presence of azole resistance mutations are commercially available [14]. In humans such assays enabled the detection of azole-resistant *A. fumigatus* directly in BAL-fluid [14,15]. The *A. fumigatus* strain recovered from our case harbored a resistance mutation that is associated with environmental resistance selection [5], indicating that the dolphin inhaled *A. fumigatus* conidia that were already azole-resistant.

Treatment options in humans and animals with azole-resistant IA are limited. Experts recommended to move away from azole monotherapy when resistance is documented, switching to liposomal amphotericin B or voriconazole and echinocandin combination therapy [16]. In vitro and in vivo studies indicate that the activity of polyenes and echinocandins are not affected by the presence of azole resistance mutations [17–19]. Furthermore, voriconazole and anidulafungin combination therapy was shown to be effective in an animal model of disseminated IA due to *A. fumigatus* with low-level voriconazole resistance (MIC of 4 mg/l). However, the efficacy of the combination against voriconazole high-level resistant *A. fumigatus* has not been studied [18]. There is concern that voriconazole will not be effective in high-level resistant *A. fumigatus* infection and combination therapy will solely rely on anidulafungin efficacy, which is suboptimal [18].

As intravenous therapy was no option in treating the dolphin, it was decided to increase the exposure of posaconazole. Using results of pharmacokinetic and pharmacodynamic in vitro and in vivo models we have previously attempted to bridge these experimental results to human infection by calculating which posaconazole exposure would be required in relation to the *A. fumigatus* MIC to treat successfully [8]. For each MIC the corresponding posaconazole exposure and plasma level were determined [8]. Based on our analysis a posaconazole plasma-level of 3.09–3.33 mg/l would be required to treat an infection with a posaconazole MIC of 0.5 mg/l and 6.18–6.66 mg/l for a MIC of 1.0 mg/l [8]. Indeed when a plasma level of > 3 mg/l was achieved in the dolphin, gradual clinical response was documented without significant toxicity ultimately leading to clinical cure.

In the dolphin very high drug exposures were achieved using the oral solution formulation, but in humans posaconazole drug levels above 3 mg/l could not be achieved due to limited bioavailability associated with the oral suspension. However, phase I and phase II pharmacokinetic studies indicate that a posaconazole plasma level above 3 mg/l can be achieved using the new intravenous and tablet formulations, without significant side-effects [20]. As the majority of azole-resistant *A. fumigatus* isolates show low-level resistance to posaconazole, i.e. within the 0.5 mg/l to 1 mg/l MIC range [17], intravenous posaconazole might be a treatment option in human cases of azole-resistant IA.

Our study shows proof-of-principle of high-dose posaconazole for the treatment of IA due to posaconazole low-level-resistant *A. fumigatus*. With the availability of new posaconazole formulations further studies need to explore this option for the management of azole-resistant IA in animals and humans.

Conflict of interest

P.E. Verweij has received research grants and served as consultant for Gilead Sciences, Pfizer, MSD, Astellas, F2G, and Basilea. Other authors: 'there are none'.

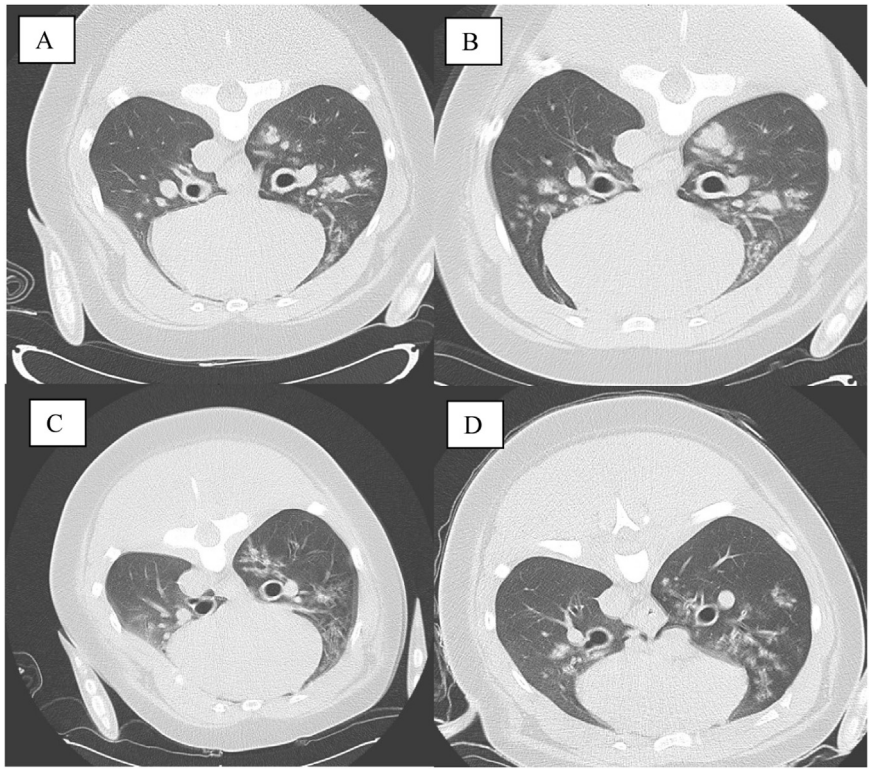

Fig. 2. Evolution of pulmonary lesions on consecutive CT-scans of the lung at the level of the heart. Panel A, Axial CT image, taken before high-dose posaconazole treatment was started, showing small granulomas and some infiltrates at the left side; Panel B, At 6 weeks of high dose posaconazole therapy. The CT image shows a progression of the granulomas at the left side and some infiltrates at both sides; Panel C, After 14 weeks of posaconazole high-dose therapy, showing a substantial reduction of the granulomas and infiltrates; Panel D, CT imaging after 22 weeks of high-dose posaconazole treatment showing complete resolution of the granulomas. Some residual peribronchial lesions are visible.

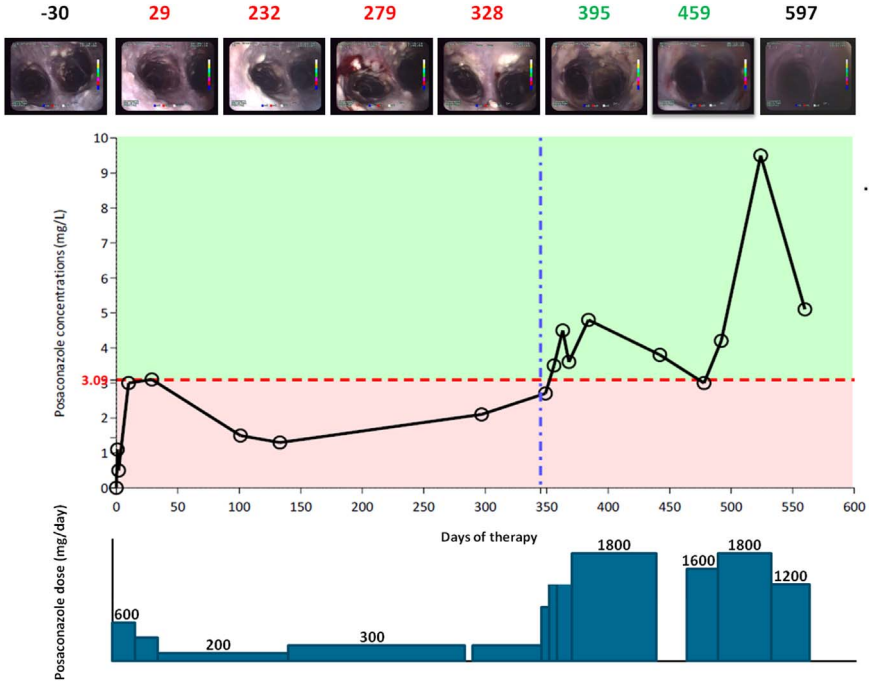

Fig. 3. Posaconazole dose (lower panel), posaconazole trough plasma levels (mg/l; middle panel) and consecutive bronchoscopy images of *Aspergillus* trachea lesions (upper panel). Green indicates the area of posaconazole exposure calculated to be sufficient to successfully treat posaconazole low-resistant *A. fumigatus* (MIC 0.5 mg/l), while the red area represents insufficient posaconazole exposure. Day 0 indicates the first day of posaconazole therapy. Posaconazole treatment was interrupted for 11 days (day 445–455) because the dolphin was clinically poor and a possible side effect of high-dose posaconazole needed to be ruled-out. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Acknowledgements

We thank Henrich van der Lee and Ton Rijs for their technical support.

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