Siderophore-based Monitoring of Posaconazole Therapy in a Rat Model of Invasive Pulmonary Aspergillosis

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Introduction

Invasive pulmonary aspergillosis (IPA) is a rapidly progressing opportunistic fungal infection caused by *Aspergillus fumigatus* in immunocompromised patients [1]. As iron is tightly regulated within humans [2], this pathogen produces two extracellular (fusarinine C, triacetylfusarinine C) and two intracellular (ferricrocin, hydroxyferricrocin) siderophores, enabling the iron acquisition and storage [3]. Next, secreted mycotoxin gliotoxin exerts detrimental effects on several types of mammalian immune cells [4]. Most of the current diagnostic methods of IPA are time-consuming, non-specific, invasive, and can contribute to high mortality rates (30–95 %) [5]. We previously showed that siderophores could play the role of early and specific markers of IPA [6]. This work aims to distinguish the disease progression between treated and untreated rats by simultaneous detection of siderophores, gliotoxin, antifungal posaconazole, and their metabolites (**Fig. 1**) from non-invasively collected urine.

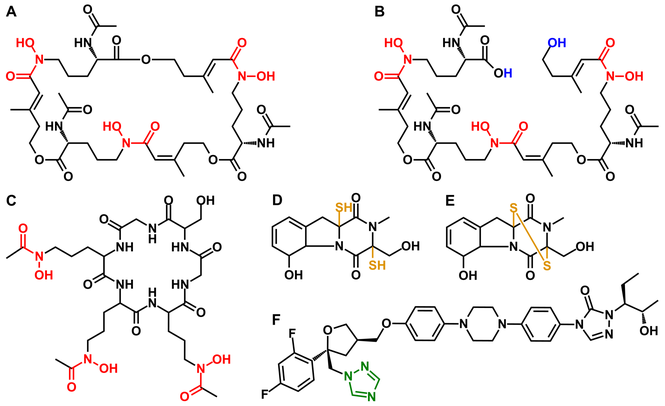

Fig. 1 The extracellular siderophore triacetylfusarinine C (TAFC; A) forms its degradation product triacetylfusarinine B (TAFB; B) after hydration of the ester bond (in blue). Hydroxamate groups (in red) chelate ferric cation in both TAFC, TAFB, and intracellular siderophore ferricrocin (FC; C). Disulfide bridge formation (in orange) in gliotoxin (GTX; D, E) generates deleterious reactive oxygen species. The fungicidal effect of posaconazole (POS, F) arises after interaction between nitrogen in triazole core (in green) and heme iron in lanosterol 14-alpha demethylase, one of the enzymes responsible for ergosterol biosynthesis.

References

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Experiment

Eight female Lewis rats were infected intratracheally with the fungal strain *A. fumigatus* 1059 CCF (100 µL, 10^7 spores). Neutropenia was induced by two intraperitoneal doses of cyclophosphamide (75 mg/kg). Four rats received a multidose posaconazole therapy *per os* (4 mg/kg/day), which started three days post-inoculation. If possible, urine was collected twice a day, and lungs and blood were taken after sacrificing the rats. Samples underwent double liquid-liquid extraction with ethyl acetate and consequent protein precipitation with pre-cooled methanol. The simultaneous detection of analytes was performed using a Dionex UltiMate 3000 HPLC system connected to a SolariX 12T FTICR mass spectrometer in electrospray ionization positive-ion mode. Quantification was performed using a matrix-matched calibration or standard addition method.

Results

IPA monitoring in the untreated rats (Fig. 2)

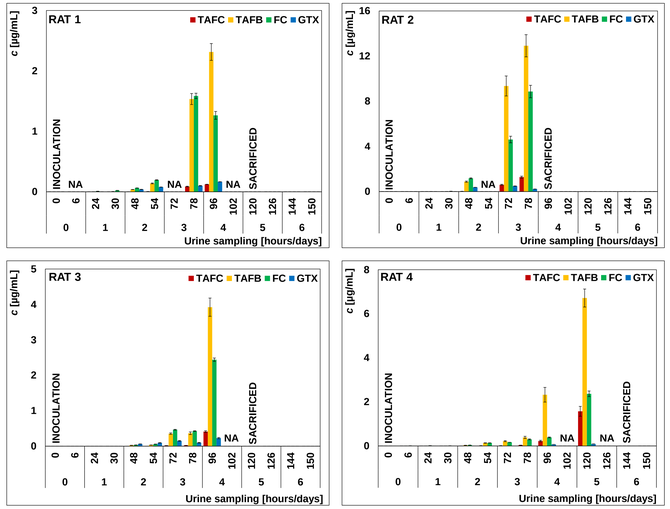

Fig. 2 Cyclophosphamide induced neutropenia favoured the germination of *A. fumigatus* conidia to complete fungal hyphae, activating the secondary metabolism as reflected by urinal detection of TAFC, TAFB, FC, and GTX. We firstly detected these biomarkers between 24–48 hours after inoculation. Further concentration increase of these biomarkers mirrored the severity of ongoing aspergillosis and, together with the lack of posaconazole intervention, all rats had to be sacrificed. NA: not available.

Contact

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IPA monitoring in the treated rats (Fig. 3)

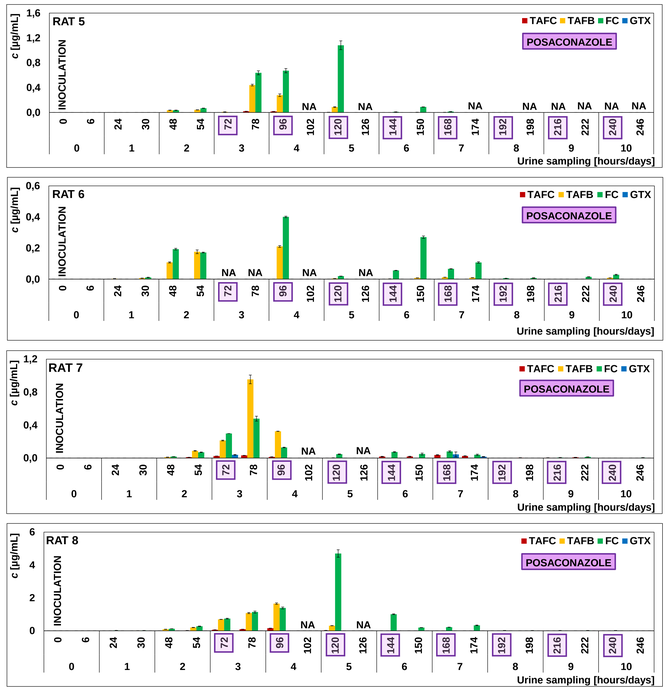

Fig. 3 Starting three days post-infection, POS was administered every 24 hours *per os*. Posaconazole reaches its maximal plasma concentration between four and six hours [7]; therefore, in urine, its fungicidal effect began to be observed 24 hours after the first dose application reflected as concentration decrease of extracellular TAFC and TAFB, followed by intracellular FC. This may indicate the shutdown of siderophore biosynthesis with the consequent release of intracellular hyphae content into the rat body. NA: not available.

Biomarkers in lungs and blood (Fig. 4)

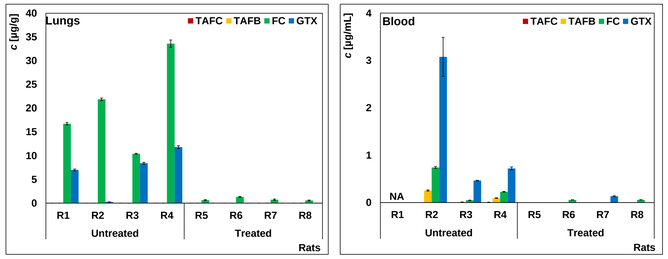

Fig. 4 Besides urine, we collected lungs (left) and blood (right) after rat sacrifice. POS preferably accumulates in the alveoli [7]; therefore, intracellular FC represents the key metabolite showing the extent of fungal burden. The presence of FC in the treated rats points to substantial reduction but not complete eradication of *A. fumigatus*. Gliotoxin detection in the untreated rats (representing the main circulating metabolite in the blood) may refer to the partial recovery of immune response in the alveoli, as GTX suppresses the function of alveolar macrophages. Compared to blood, neither TAFC nor TAFB had been detected in the lungs. NA: not available.

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Conclusions

We provided the first kinetic insight into the biosynthesis of triacetylfusarinine C, ferricrocin, and gliotoxin affected by multidose posaconazole therapy in a neutropenic rat model of invasive pulmonary aspergillosis.

We confirmed the utilization of siderophores as early virulence factors thanks to urinal detection of triacetylfusarinine C, its main degradation product triacetylfusarinine B, and ferricrocin between 24 and 48 hours post-inoculation.

In urine, posaconazole fungicidal effect resulted in prompt triacetylfusarinine C attenuation, followed by delayed clearance of intracellular ferricrocin. This may indicate the extent of hyphae lysis, thus showing the antifungal treatment efficiency.

Selective accumulation of posaconazole within the alveoli affected the presence of intracellular ferricrocin, indicating considerable reduction but not complete eradication of *Aspergillus fumigatus*.

The restored gliotoxin biosynthesis may refer to partial recovery of the alveolar immune response in the untreated rats, as the neutropenia was induced by two consecutive cyclophosphamide doses five and one days before inoculation.

Our LC-FTICR-MS-based approach allows an early and non-invasive tracking of invasive pulmonary aspergillosis. Concurrent detection of antifungals and siderophores may provide an insight into the efficiency of antimycotic therapy, thus reducing the treatment time, drug-related toxicities and adverse effects.