Fighting (for iron) to survive: therapeutic avenues for aspergillus fumigatus infections

Aspergillus fumigatus (Af), a common fungus, frequently infects the lungs of patients with compromised immune systems. Despite progress in developing anti-fungal agents, *Af* infections still cause a significant number of deaths, making new treatment options necessary to reduce the disease burden.

Why does *Af* cause such aggressive infections? One factor comes from its ability to form biofilms. *Af* biofilms consist of an aggregate of the fungus attached to a surface. Once established, the biofilm protects itself by creating a shield that isolates it from the external environment, including anti-fungal drugs. Because *Af* can form biofilms during human infections, a new treatment possibility for *Af* is to inhibit biofilm survival.

Iron is a critical nutrient for *Af*. Iron deprivation can inhibit its survival, and a class of molecules, known as iron chelators, can accomplish that task. They hide iron from the *Af* and prevent the fungus from using it. However, the effects of iron deprivation on the *Af* when it is growing as a biofilm are not known. Thus, to provide new potential treatment avenues for *Af* infections, we studied how chelators effect *Af* biofilm growth.

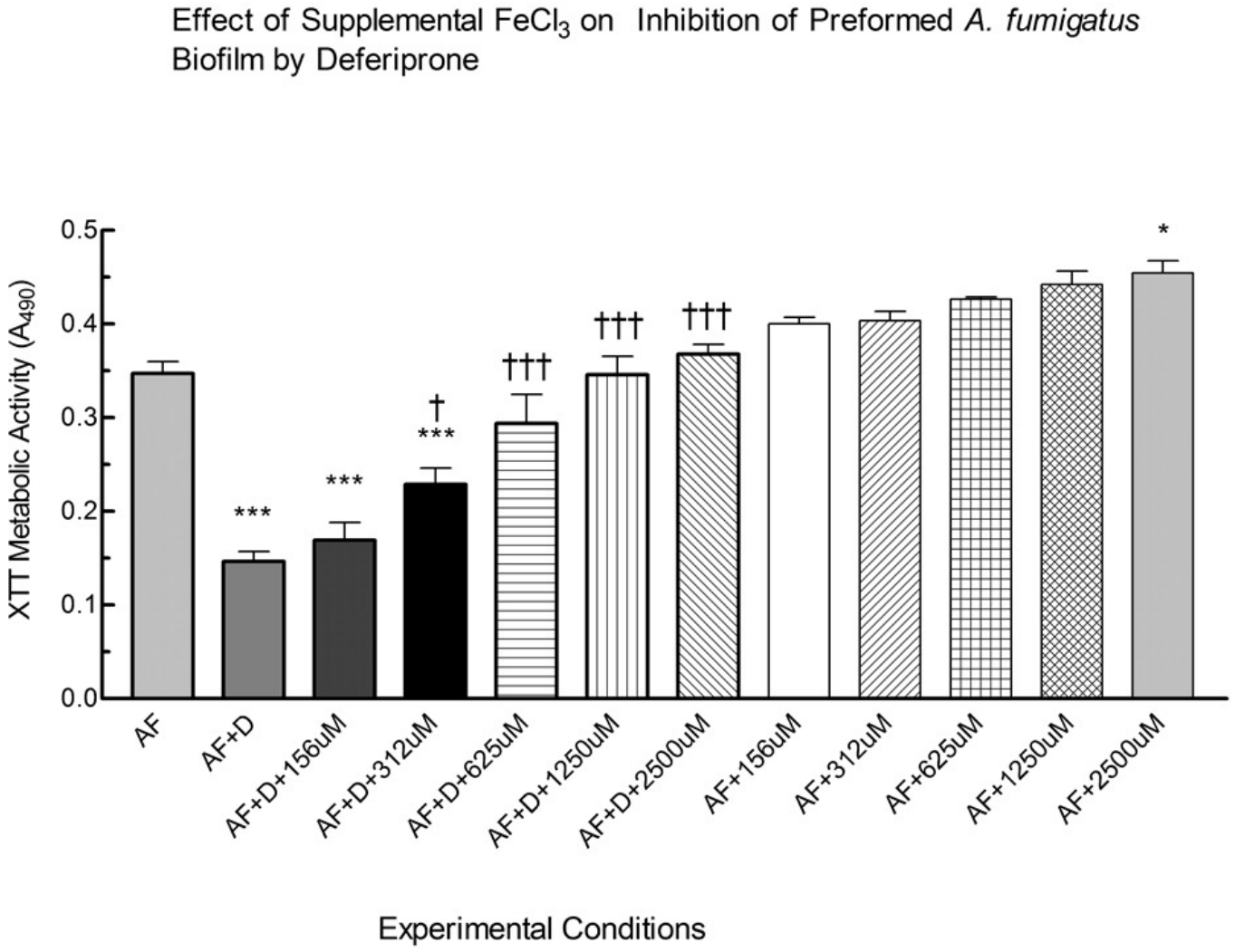

Fig. 1: Excess iron's role in reversing DFP Inhibition of Af biofilm. AF=Aspergillus, D=DFP, the numbers represent the iron concentration in micromolar amounts. DFP concentration = 625µM. Asterisks designate a significant difference compared to Af alone. Daggers represent a significant difference compared to AF + D. Note the stepwise increase in biofilm activity as the added iron amounts increase

We tested three iron chelators, Deferasirox (DFS), Deferiprone (DFP), and Deferoxamine (DFM) against *Af* in 3 growth conditions:

Not forming biofilm- growing, but with no surface for attachment.
Forming biofilm - trying to attach and start the biofilm development,
Preformed biofilm- continuing to grow after establishing a biofilm.

The first question we asked was, “Could chelators inhibit the *Af* in these growth conditions?”

In condition 1, we found DFP and DFS inhibit *Af* at the highest concentrations we tested, while DFM did not. Since DFP and DFS had similar effects in condition 1, we decided to use DFP in conditions 2 and 3. Here, we found DFM had no effect on *Af* biofilms, except in the highest concentration we tested. At that level, it actually *enhanced* the biofilm, making DFM an undesirable option for treatment. There is evidence that DFM, instead of keeping iron away, forms with iron a package that *increases* iron uptake in the fungus.

DFP, however, was effective in preventing the formation and further development of *Af* biofilm. When the *Af* was trying to form biofilm, the highest concentrations of DFP reduced the *Af* activity by about 70%. When the *Af* had already formed a biofilm, it was more resistant to DFP. Nevertheless, the highest concentrations still reduced the biofilm activity by up to 35%.

Despite this finding, we still needed to know whether DFP’s effects are due to its impact on access to iron, or another factor. To answer this question, we added varying amounts of extra iron to the *Af’s* environment with and without DFP. If the DFP inhibits the *Af* by binding iron, then adding more iron should overcome the inhibition by DFP.

Figure 1 shows this. When the *Af* was given extra iron and DFP, the DFP continued to inhibit until the increasing amounts of iron added reversed the DFP effect. This gives us confidence that DFP acts by impacting *Af’s* access to iron.

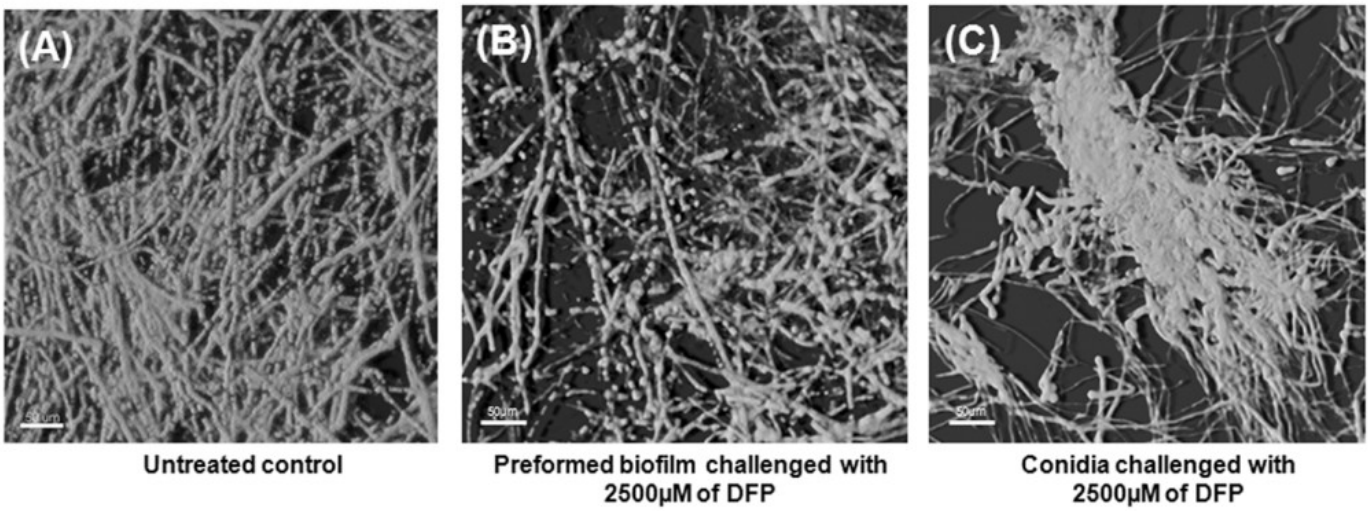

Fig. 2: Microscopic images showing DFP’s reduction in biofilm density and organization

While the decrease in Af activity is encouraging, we wanted to be sure that the DFP was actually

inhibiting the overall architecture of the biofilm. To examine this, we used a special computerized microscope to photograph the biofilm with and without DFP (Fig. 2). These photos show DFP reduces the organization and density of the biofilm.
In summary, some chelators represent a potential treatment option for *Af* infections as they can inhibit biofilm formation and growth. This represents one step on the path to finding new treatments that can help reduce the burden of *Af* disease.

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Publication

Effects of Iron Chelators on the Formation and Development of Aspergillus fumigatus Biofilm.
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