P003

Synthetic antifungal peptide mimic kills *Candida albicans* by targeting protein glycosylation and synergistically prevents infection

Sebastian Schaefer[1,2,4], Raghav Vij[2], Jakob Sprague[2], Eric Seemann[3], Bernhard Hube[2], Megan Lenardon[4], Cyrille Boyer[1], Sascha Brunke[2]
[1]School of Chemical Engineering, University of New South Wales, Sydney, Australia
[2]Department of Microbial Pathogenicity Mechanisms, Hans Knoell Institute, Jena, Germany
[3]University Hospital Jena, Jena, Germany
[4]School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, Australia

Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Fungal infections represent a serious burden on human health. Increasing numbers of susceptible hosts, a limited set of approved antifungal drugs which frequently trigger undesired side effects, and the emergence of resistant strains highlight the urgent demand for novel antifungal drug formulations. However, the biological similarity of human and fungal cells hampers the development of new antifungals which do not also harm humans. In nature, organisms in almost all domains of life produce antimicrobial peptides to combat microbial pathogens. Those peptides share certain characteristics, such as being short, amphiphilic molecules with a positive net charge.[1]

We designed synthetic polyacrylamides which mimic the properties of naturally occurring antifungal peptides. These positively charged, amphiphilic polymers are advantageous over peptides because of their easy synthesis and stability against proteases. Initial structure-activity relationship studies revealed an optimal cLogP (the calculated hydrophobicity of a molecule) around 1.5 to ensure activity against *C. albicans* and simultaneous biocompatibility with host cells.[2] Additionally, shorter polymers with a length of 20 subunits were more effective than their longer versions.[2] In terms of their therapeutic index, certain compositions outperformed the broad-spectrum antifungal amphotericin B and were even effective against drug-resistant clinical isolates of *C. albicans*.[2]

Candida albicans strains with known antifungal drug-resistance mutations were not affected in their susceptibility to the polymers. Therefore, investigations were carried out to elucidate the mode of action of the polymers. The transcriptome of *C. albicans* cells treated with subinhibitory concentrations of the polymers revealed an increased expression of genes involved in general stress response and upregulation in protein processing in the endoplasmic reticulum, particularly glycosylation and degradation. These findings, together with electron microscopy observations, indicated damage to the mannoproteins in the cell wall of the fungus. Membrane damage was also observed utilizing a *C. albicans* strain expressing GFP intracellularly.

The *in vitro* therapeutic potential of the most promising polymer was tested in a human epithelial cell (HEC) model simulating *C. albicans* infection. The polymer alone was not able to prevent *C. albicans* infection of HECs. However, the combination of polymer with caspofungin or fluconazole showed very strong synergistic effects at otherwise non-inhibitory concentrations of the individual antifungals, successfully stopping fungal infection in vitro without damaging the HECs.

These results underline the potential of synthetic polymers as an alternative treatment for fungal infections with low toxicity to human cells and a novel mode of action.

Sources:

1. Fernández de Ullivarri, M., Arbulu, S., Garcia-Gutierrez, E. and Cotter, P.D. Antifungal peptides as therapeutic agents. Front Cell Infect Microbiol 10, 00 105 (2020).

2. Schaefer, S. et al. Rational design of an antifungal polyacrylamide library with reduced host-cell toxicity. ACS Appl Mater Interfaces 13, 27430-27444 (2021).

P004

Neglected risk for of invasive candidiasis: s tudy of distribution, species differentiation and antifungal susceptibility pattern of Candidemia among patients with liver disease

Pradeep Kumar, Mohd Ayyub, Pratibha Kale
Department of Clinical Microbiology, Institute of Liver and Biliary Sciences, New Delhi, India

Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Objectives: Patients with liver disease (LD) are more predisposed to candidemia due to the dysfunctional Kupffer cells which fails to capture the circulating yeasts, thereby causing fungal dissemination. Candidemia is ten times more common among end-stage liver disease patients compared with other LD patients. Compared to those with abdominal candidiasis, ICU admission and mortality are found to be higher in liver transplant recipients (LTRs) with candidemia. Though a majority of *Candida* infection among LD patients are due to *C. albicans*, there has been an increasing prevalence of non-*albicans* isolates. This study was mainly aimed at species differentiation and antifungal susceptibility. Our objective was to study the pattern of *Candida* isolates from blood among patients with different LD states admitted to tertiary care Liver Hospital. To determine the distribution of Candidemia in patients with different liver disease states. To determine the various species of *Candida* isolated from blood in patients with liver diseases. To determine the anti-fungal susceptibility pattern of *Candida* isolates in blood in patients with liver disease. To determine the year-wise changing trends in *Candida* spp. isolated in blood and its anti-fungal susceptibility pattern in patients with liver disease.

Materials and Methods: This is a retrospective observational study conducted in the department of Microbiology at Institute of Liver and Biliary Sciences (ILBS), New Delhi. Ethical approval for this study (IEC/2021/84/NA06) was provided by the Institutional ethics committee/Institutional review board. A total of 118 LD patients with candidemia who were admitted to ILBS between January 2017 to December 2020 were included in the study. Clinical details of these patients were collected from the hospital information system. Patients with LD were divided into 4 groups—acute liver failure (ALF), acute on chronic liver failure (ACLF), chronic liver disease (CLD), and post-liver transplantation (Post-LT). *Candida* speciation as identified by VITEK® 2 (Biomérieux) and also its antifungal susceptibility pattern by broth microdilution.

Results: The mean age of the 118 LD patients was 48.6 ± 14.9 years [± standard deviation (SD)]. Among them 107 (90.7%) were males and 11 (9.3%) were females. Among the 118 LD patients, 6 (5.1%) were ALF patients, 32 (27.1%) were ACLF patients, 66 (55.9%) were CLD patients and 14 (11.9%) were post-LT patients. The most common *Candida* species isolated was *C. tropicalis* (22.9%), followed by *C. glabrata* (17.8%), *C. albicans* (16.9%), *C. parapsilosis* (12.7%), *C. auris* (8.5%), *C. krusei* (6.8%) and other *Candida* species (14.4%). The other *Candida* species isolated were *C. haemulonii*, *C. kefyr*, *C. lusitaniae*, *C.pelliculosa*, *C. duobushaemulonii*, *C. lipolytica*, and *C. rugosa*. The sensitivity to fluconazole (55.1%), voriconazole (86.4%), caspofungin (82.2%), micafungin (97.5%), flucytosine (80.5%), amphotericin B (86.4%). Mortality was 66%.

Conclusion: Our study shows that chronic liver disease, acute liver failure and liver transplantation are important risk factors for invasive candidiasis. There was a rise in the non-*albicans Candida*, especially *C. tropicalis* and *C. glabrata*. There is rising antifungal resistance to azoles and can lead to therapy failure. Hence antifungal sensitivity testing is essential in patients with these neglected risk factors to prevent mortality.

P005

Competitive fitness and trade-offs associated with azole resistance in *Candida auris* clinical isolates

Sourav Das[1], Yamini Tawde[1], Shreya Singh[1], Arunaloke Chakrabarti[1], Shivaprakash M Rudramurthy[1], Harsimran Kaur[1], Tushar Shaw[2], Anup Ghosh[1]
[1]Department of Medical Microbiology. Post Graduate Institute of Medical Education and Research, Chandigarh. India, Chandigarh, India
[2]Department of Life and Allied Health Sciences, Ramaiah University of Applied Sciences., Bengaluru, India

Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Objectives: Following the global emergence of *Candida auris*, this multidrug-resistant yeast has become a concern of serious threat to public health. The evolution of such multidrug-resistant pathogens depends on their relative fitness to their susceptible counterparts. Fitness cost, expressed in terms of reduced competitive ability of survival in the absence of drugs, plays a key role in the drug resistance dynamics. The objectives of the study were to investigate the oxidative stress response (OSR) in fluconazole-resistant *C. auris* and to compare its relative fitness with fluconazole-susceptible strains.

Methods: A total of 351 *C. auris* clinical isolates (61 flu-susceptible and 290 flu-resistant) were screened for stress tolerance by spot assay on yeast-peptone-dextrose (YPD) agar containing 5-50 mM H2O2 (with 5 mM increments) and results were rechecked on YPD broth. Expressions of Hog1, Cta1, Sod1, Mkc1, Cek1, Calcineurin1 genes were evaluated under oxidative stress by qPCR. Cellular catalase level was determined by colorimetric catalase assay kit (EnzyChrom, Bioassay systems). DHR-123 assay was performed to measure the intracellular reactive oxygen species level (iROS) under H2O2 exposure. Adherence, biofilm formation and XTT [2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-%-Carboxanilide] cell viability were estimated under hyper-oxidative stress condition. *C. auris* were co-cultured with human neutrophils from healthy donors and the percentage of fungal killing was recorded. Scanning electron microscopy (SEM) images were taken to check the cellular association under increasing H2O2 exposure.

Results: A total of 95.08% (58/61) flu-susceptible isolates were hyper-resistant to oxidative stress (30-50 mM) while 94.5% (274/290) flu-resistant isolates showed lower tolerance to oxidative stress (10-25 mM). Hog1 ($P = .0012$) and Cta1 ($P = .01$) transcript levels were highly elevated in flu-susceptible isolates when exposed to 10 mM of H2O2 while no significant difference was observed in resistant isolates. Flu-susceptible isolates exhibited a higher level of catalase ($P = .002$) compared with resistant isolates upon 10 mM H2O2 exposure. Owing to the reduced catalase activity, a higher iROS level was accumulated in resistant isolates. Flu-susceptible isolates formed higher biofilms at 37°C both under no-stress (control, $P < .001$) and hyper-oxidative condition (10 mM, $P = .002$). At 42°C, there was a significant reduction in biofilm among resistant isolates under 10 mM H2O2 compared to no-stress control (from mean OD 0.48 ± 0.26 to 0.23 ± 0.09) ($P = .02$). Susceptible isolates had better adherence capacity even at 42°C both under no-stress (control, $P = .01$) and under 15 mM H2O2 exposure ($P < .001$). Higher fungal killing was achieved in Flu-resistant isolates when co-cultured with human neutrophils ($P = .014$) compared to susceptible counterparts.

Conclusion: Collectively, these data revealed that in *C. auris*, resistance to fluconazole is accompanied by fitness trade-offs. Fluconazole-resistant *C. auris* strains have diminished ability to survive the diverse array of stresses that are encountered in a mammalian host and reduced virulence traits essential for invasive disease.

P006

Resistance of *Aspergillus flavus* clinical isolates and associated fitness-cost

Elie Djenontin[1,5], Anne Debourgogne[1], Imane Bergui[2], Jean-Marc Costa[3], Bita Mousavi[1], Nawel Ait-Ammar[1,4], Jacques Guillot[1], Françoise Botterel[1,4], Eric Dannaoui[1,2,5]
[1]Dynamyc Upec, Enva, Usc Anses, Faculté De Médecine De Créteil, Créteil, France
[2]Université Paris Descartes, Faculté de Médecine, Paris, France
[3]Laboratoire CERBA, Saint Ouen L'Aumône, France
[4]AP-HP, Hôpital Universitaire Mondor, laboratoire de Parasitologie-Mycologie, Créteil, France
[5]AP-HP, Hôpital Européen Georges Pompidou, Unité de Parasitologie-Mycologie, Paris, France

Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Objectives: *Aspergillus flavus* and closely related species could be pathogenic for humans, animals, and plants and could also produce mycotoxins. The members of the Flavi section are morphologically quite similar making precise identification to the species level difficult. In this study, we present the antifungal susceptibility profiles of French clinical isolates belonging to the Flavi section. Isolates have been characterized by molecular methods and the potential fitness-cost associated with azole-resistance has been determined.

Methods: A total of 120 isolates phenotypically identified as *A. flavus* were included in the study. These clinical isolates were recovered over a 15-year period (2001-2015). For all isolates, specific identification was confirmed by sequencing a part of the β-tubulin and calmodulin genes. The isolates were first screened for their susceptibility to azoles antifungal agents by using 3-sectors agar plates containing itraconazole, voriconazole, and a drug-free control. Susceptibility to six antifungal drugs was further determined by using the EUCAST reference microdilution broth technique. Fitness cost was evaluated by growth curve kinetics in RPMI and by evaluation of virulence in a Galleria mellonella invertebrate animal model.

Results: Out of 120 isolates, molecular analysis of the partial β-tubulin and calmodulin sequences showed that 117 isolates were *A. flavus sensu stricto* and the three remaining corresponded to *A. parasiticus*, *A. nomius*, and *A. tamarii*. Two isolates were azole-resistant by the screening test. For the *A. flavus sensu stricto* isolates, the geometric mean MIC values (range) of amphotericin B, itraconazole, voriconazole, posaconazole, isavuconazole, and caspofungin were 1.84 (0.25-16), 0.29 (0.125-2), 0.82 (0.5-8), 0.27 (0.06-2), 1.15 (0.25-8), and 0.061 (0.03-0.125) μg/ml, respectively. For *A. parasiticus*, *A. nomius*, and *A. tamarii*, MICs were in the same range. Two *A. flavus sensu stricto* isolates (AfR1 and AfR2) had voriconazole and isavuconazole MICs at 8 μg/ml. Compared to susceptible isolates, these two azole-resistant isolates had a delayed growth in RPMI liquid medium. In the *G. mellonella* model, the mortality was 100% for susceptible isolates. In contrast, the Galleria infected by AfR1 and AfR2 showed a significantly lower mortality rate.

Conclusion: Antifungal susceptibility to six drugs was determined on a large collection of clinical isolates belonging to *Aspergillus Flavi* section. Most of the isolates were identified as *A. flavus sensu stricto* and most of them were susceptible to antifungal drugs. Nevertheless, the occurrence of two resistant isolates highlights the need for susceptibility testing for *A. flavus*. It seems that azole-resistance is associated with a fitness-cost including a lower growth rate and a lower virulence.

P007

Fumaric reductase analog regulates sensitivity of *pyrvinium pamoate* and voriconazoole against *Exophiala Dermatitidis*

Xiaotong Li[3], Yutang Zhu[3], Jie Zhang[3], Yi Sun[2], Lujuan Gao[1]
[1]Fudan Universiry Zhongshan Hospital, Shanghai, China
[2]Jingzhou Central Hospital, Jingzhou, China
[3]Yangtze University, Jingzhou, China

Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Objectives: The black yeast *Exophiala dermatitidis* is an opportunistic pathogen that causes phaeohyphomycosis in both immunocompetent individuals and immunosuppressed patients, resulting in localized cutaneous and subcutaneous infections to more severe systemic forms such as neurotropic infections. Besides, *E. dermatitidis* was frequently found as a colonizer of pulmonary of cystic fibrosis patients, which appears to be associated with more advanced disease. Infections of *E. dermatitidis* are often chronic and recalcitrant. Previously, we have demonstrated that pyrvinium pamoate (PP) exerted antifungal activity alone (MIC 2 μg/ml) and favorable synergy with azoles against *E. dermatitidis in vitro*, which was confirmed *in vivo* via *Galleria mellonella* model. Further investigation revealed pyrvinium resulted in significant growth restriction of *E. dermatitidis*, reduction of biofilm formation, and significantly ($P < .05$) decrease of the efflux of Rhodamine 6 G. However, the underlying mechanism and probable target of PP are still unknown. The aim of this study is to investigate the role of fumaric reductase analog in the effect of PP against *E. dermatitidis*.

Methods: The knockout strain named ΔExfr was constituted via polyethyleneglycol (PEG)-mediated transformation of protoplasts. A gene replacement cassette was generated as described. *E. dermatitidis* wild-type strain ATCC34100 DNA was used for molecular cloning. The pan7-1 plasmid vector, which contained the genetic markers hygromycin B phosphotransferase, was applied as a selection marker provider. DNA manipulation was performed according to standard laboratory procedures. The 5′ and 3′ flanking regions of fumaric reductase analog coding gene Exfr (Genbank geneID 20312383) were amplified using the primers ExfruF, ExfruR, and ExfrdF, ExfrdR, respectively. The selection maker Hph was amplified from pan7-1 by using primers hphF and hphR. These three fragments are subsequently fused via PCR with primers ExfuF and ExfuR. The protoplasts of wild-type strain ATCC34100 were prepared as described. Further, the fused gene fragment and PEG was mixed with protoplast to complete transformation, and the transformants were transferred to SDA solid culture base (containing a final concentration of 50 μg/ml hygromycin). DNA extracted from the transformants that could grow stably on HPH medium was sent for sequencing to verify that the target gene had been introduced into the location. The growth, morphology, and *in vitro* susceptibility of ATCC34100 and ΔExfr were further investigated.

Results: There was no significant difference in the colony or conidial morphology. However, the growth rate of ΔExfr was slightly slower than that of the wild-type strain. In addition, the MICs of pyrvinium pamoate (PP) and voriconazole against ΔExfr were 2-fold higher (16 ug/ml and 0.5ug/ml, respectively), compared with that of the wild-type strain.

Conclusion: The preliminary results suggested that fumaric reductase analog may play an important role in drug sensitivity of *E. dermatitidis*, especially the sensitivity to PP and voriconazole. In addition, ΔExfr is currently the only *E. dermatitidis* strain that was successfully knocked out by protoplast procedure, which provides reference for subsequent researches on *E. dermatitidis* gene knockout.