MAP kinases (p38, ERK, JNK) resulted in decreased cytokine production after stimulating PBMCs with *C. albicans* or *C. parapsilosis*, indicating that these kinases are all involved in the signal-transduction following the recognition of the two *Candida* species. However, cytokine levels indicated that there are certain differences in the signal-transduction following the immune sensing of *C. albicans* and *C. parapsilosis* by PBMCs. Additionally, decreased cytokine production following the inhibition of Dectin-1 revealed that this receptor plays a role in the recognition of both *C. albicans* and *C. parapsilosis*. To further elucidate the immune responses evoked by *C. parapsilosis*, we examined the interactions of this pathogen with human primary monocytes-derived macrophages. As secreted fungal lipases have been shown to play in important role an pathogenesis, we compared the response of human macrophages to a wild type (wt) as well as a lipase deficient ($lip^{-/-}$) *C. parapsilosis* strain that has been previously established in our lab. When co-cultured with macrophages, both strains induced a significant increase in the expression of TNFα, IL-1β, IL-6, IL-8 and PTGS-2 (prostaglandin-endoperoxide synthase 2) genes in host cells after 12 hours, as determined by quantitative real-time PCR. Notably, macrophages stimulated with lipase mutant *C. parapsilosis* showed at least two-fold higher expression of these pro-inflammatory mediators compared to those infected with lipase-producing (wt) *C. parapsilosis*. Additionally, we examined the phagocytosis of wt and $lip^{-/-}$ *C. parapsilosis* strains by human PBMC-derived macrophages using quantitative imaging flow cytometry. We found that although after 2 hours both strains were phagocytosed to the same extent by host cells, the rate of internalization and phagolysosome fusion was higher in case of $lip^{-/-}$ *C. parapsilosis*. These findings confirm the role of fungal lipases as important virulence factors during *C. parapsilosis* infection and support the hypothesis that these microbial compounds have anti-inflammatory potential. Taken together, our results contribute to the better understanding of the immune response induced by *C. parapsilosis*, and highlight the role of fungal lipases during host-pathogen interactions.

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Investigating the structure and the mechanism of action of *Neosartorya fischeri* antifungal protein

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Today there is a substantial demand for new antimicrobial compounds because of the increasing number of fungal infections. The defensin-like antimicrobial peptides produced by filamentous fungi are interesting in this respect, because they can inhibit the growth of several filamentous fungi. Different defensin-like antimicrobial miniproteins have been isolated from seven ascomycetous filamentous fungal species (*Aspergillus clavatus*, *Aspergillus giganteus*, *Aspergillus niger*, *Fusarium polyphialidicum*, *Neosartorya fischeri*, *Penicillium chrysogenum*, *Penicillium nalgiovense*). Until now the *Penicillium chrysogenum* antifungal protein (PAF) and the *Aspergillus giganteus* antifungal protein (AFP) have been intensively studied. Although AFP and PAF generate similar symptoms in the susceptible organisms, they have different mode of actions. AFP disturbs the cell wall biosynthesis by specific inhibition of chitin synthases, and PAF evokes programmed cell death via G-protein signal transduction pathway. The *Neosartorya fischeri* antifungal protein (NFAP) consists of 57 amino acid residues and has a calculated molecular mass of 6625.5 Da and a pI of 8.93. Our *in silico* structure modelling revealed that NFAP contains five antiparallel β-strands connected by three loops, and showing a β-barrel topology in general, which is stabilized by three intramolecular disulfide bridges. Previously we demonstrated that NFAP effectively inhibits the growth of numerous filamentous fungi including human and plant pathogens and the model organism, *Aspergillus nidulans*. As in the case of similar proteins, the high yield production of NFAP is not resolved despite the available knowledge of the nature of its 5′-upstream transcriptional regulation elements in response to environmental signals and stress. For the future investigation it would be important that NFAP could be producible in a non-sensitive, easily fermentable, "generally recognized as safe" fungus. On the other hand the understanding the exact antimicrobial effect and the mode of action of NFAP are essential for its future practical applications.

For these reasons, we carried out the heterologous expression of the *nfap* gene in *Pichia pastoris* KM71H by using the pPICZαA vector. After purification, the final yield of the hNFAP from 1000 ml ferment broth was 2.4±0.2 mg, which is twofold amount compared to the native producer, *Neosartoria fischeri* NRRL 1881. N-terminal sequencing experiments revealed that the first 5 amino acid residues of the purified heterologous protein is LEYKG (which corresponds well to the determined amino acid sequence of the native NFAP) and the different ion chromatograms from the mass spectrometry correspond six out of all peptides found by analysis of mass lists. Based on the signal dispersion of the amide region (6-10 ppm), it is proven that the protein exists in folded state. Tertiary structure determination needs further NMR investigations using isotope-labelled NFAP. The antifungal activity of the hNFAP was the same as described in the case of NFAP. Based on our previous studies, it seems that the antifungal mechanism of NFAP differs from what was described in the case of AFP and PAF. For revealing the exact mechanism of action of NFAP fluorescent microscopic investigations and antifungal susceptibility assays on *Aspergillus nidulans* mutants in protein kinase C/mitogen-activated protein kinase and cAMP/protein kinase A signalling pathway are in progress.

Our results provide basis for further applied research, *e.g.* developing new antimicrobial peptides in therapy, pest control and food preservation.

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Opening of the blood-brain barrier for drug delivery to the brain: the effects of tesmilifene and short-chain alkylglycerols on brain endothelial cells

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The blood-brain barrier (BBB) forms a dynamic interface between the blood and the brain. It selectively regulates the transcellular and paracellular transport of molecules and passage of cells between the blood and the central nervous system. The BBB restricts drug penetration to the brain preventing effective treatment of several neurological diseases. Therefore it is an increasing need to find new ways to improve drug delivery to the brain. Brain capillary endothelial cells constitute the anatomical and functional basis of the BBB. One of the strategies to increase drug delivery to the brain is changing cerebral endothelial functions by opening the BBB through the modification of the paracellular or the transendothelial transport pathways.

In this study two agents, tesmilifene and short-chain alkylglycerols (AGs) were selected for detailed examination. Tesmilifene, a tamoxifen-related compound, has chemopotentiating properties in experimental and in clinical cancer studies. Treatment with tesmilifene caused temporary, acute CNS side-effects in patients indicating the opening of the BBB. Previous studies from our laboratory have shown that tesmilifene increases the permeability of the BBB in rats. Intraarterial injection of short-chain AGs, such as 1-*O*-pentylglycerol and 2-*O*-hexyldiglycerol, open the BBB and increase the delivery of molecules to rodent brain parenchyma *in vivo*. The mechanism underlying AG and tesmilifene-mediated modification of BBB permeability is still unknown. The aim of the present study was to test the direct effects of tesmilifene and AGs on barrier properties of cultured brain microvascular endothelial cells, a model of the BBB.

The triple co-culture BBB model was constructed on cell culture inserts using primary rat brain endothelial cells, rat cerebral glial cells and rat pericytes. Barrier integrity of the BBB endothelial monolayers was analyzed by transendothelial electrical resistance and permeability measurements. In addition to functional assays, toxicity tests, immunostainings for junctional proteins and freeze fracture electron microscopy were performed.

Short-term tesmilifene and AG treatment decreased the resistance of endothelial monolayers, and increased the permeability for fluorescein, a marker of paracellular flux. Tesmilifene also enhanced the transcellular transport of albumin. These short-term changes were accompanied by changes in cell morphology and immunostaining for junctional proteins. AG and tesmilifene-mediated increase in brain endothelial permeability was reversible. Short-term treatments did not alter the viability of brain endothelial cells. Tesmilifene did not affect the functions of P-glycoprotein, but decreased the activity of the multidrug resistance associated protein-1 and the production of nitric oxide in endothelial cells. Tesmilifene also altered the mRNA expression of several tight junction proteins measured by a custom Taqman gene array.

Our data support previous clinical observations and the results of animal experiments, and clearly indicate that AGs and tesmilifene increase the permeability of the BBB by directly acting on brain endothelial cell functions. Tesmilifene and AGs are promising adjuvants in the transient opening of the BBB for clinical use, especially for treatment of brain tumors.

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Metagenomics of biogas producing microorganisms

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The production of renewable energy carriers is currently receiving increasing attention worldwide. Biogas is a promising technology as its production may combine the treatment of various organic wastes with the generation of energy. Biogas can be converted to heat and/or electricity, and its purified derivative, biomethane, is suitable for every function for which fossil natural gas is used today. The degradation