Broadening of the antifungal pipeline by the use of polymeric nanoparticles

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The treatment options of life-threatening fungal infections are limited and need urgent improvement. The discovery of novel antifungal compounds with new mode of actions is often not the limiting factor, rather than the pharmacological properties of the compounds inhibiting the novel targets, like low water solubility, high toxicity, or low bioavailability. The use of nanocarriers can overcome these limitations, as the pharmacological characteristics of these formulations are dominated by the polymer and not by the substance itself. However, the mechanism, how polymeric nanoparticles (NPs) deliver encapsulated substances into pathogenic fungi was not fully understood. The aim of the study was to investigate, how polymeric NPs interact with pathogenic fungi, which substances can be efficiently transported by these nanocarriers and if the nanocarriers can also be used to treat intracellular persisting pathogens.

Therefore, 4 different polymers labelled with 3 different covalently attached fluorescent dyes were used to prepare the NPs. In addition, 3 different fluorescent dyes were encapsulated to track the NP and the cargo simultaneously. The interaction of the fluorescently labelled NPs with the molds *Aspergillus fumigatus*, *A. nidulans*, and *A. terreus*, or the yeasts *Cryptococcus neoformans* and *Candida albicans* was investigated by confocal laser scanning microscopy. Furthermore, the antifungal effect of the low water-soluble drug Itraconazole and the SidA inhibitor Celastrol encapsulated in these NPs was tested following the EUCAST methodology.

Irrespective of the applied conditions, none of the used NPs reached the fungal cytosol, but adhered to the fungal surface. In-depth characterization revealed that the NPs cross the fungal cell wall, but remain in invaginations of the cytoplasmic membrane. Nevertheless, encapsulation of substances lead in some, but not all, cases to a higher delivery of the compound into the fungus, pointing out also certain chemical properties, which are important for the efficient delivery by NPs. In addition, addition of NPs to macrophages prior infected with fungal pathogens resulted in colocalization of NPs with the pathogen, displaying the proof of principle that these carriers can also be used to target intracellular persisting pathogens.