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Sphingolipid targeting in inflammation and fungal infection

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Background. The sphingolipid mediator ceramide accumulates in chronic inflammatory diseases, such as Cystic Fibrosis and COPD, promoting inflammation and favoring infection. Sphingolipids (SPLs) are essential components of fungal membranes and signaling. Myriocin (Myr) inhibits SPLs synthesis in both human and fungi. We previously demonstrated that Myr loaded nanocarriers (SLN) reduces CF mice lung inflammation, ceramide content and P. aeruginosa infection (Caretti et al BBA 2014).

Hypothesis. We hypothesize that Myr modulation of SPLs synthesis, by lowering ceramide expression level, could reduce A. fumigatus infection and inflammation in both in vitro and in vivo CF models.

Methods. To assess the anti-inflammatory and antifungal potential of sphingolipids metabolism inhibition, we infected CF (IB3) and the WT (C38) human respiratory epithelial cells line with A. fumigatus w/o Myr treatment and we evaluated by RT-PCR and ELISA the cytokines expression level. Moreover, we treated CF mice model by intra-tracheal injection (by means of PennCentury micronebulisation) of SLN loaded with Myr, followed by infection with A. fumigatus, evaluating the inflammatory mediators as for airway epithelial cells. Sphingolipids analysis was performed by LC-MS spectrometry. Morphological studies on biofilm and A. fumigatus structure were done by TEM analysis while immunohistochemistry staining of lung tissue sections was used to localize and characterize A. fumigatus.

Results. We here demonstrate the antifungal activity of Myr against both planktonic A. fumigatus and preformed biofilms in vitro. TEM studies show important morphological alterations in A. fumigatus structure, such as invaginations of the cell membrane, modification in the vacuolar system and presence of multilamellar bodies, in some cases within vacuoles. From in vivo studies in murine model of pulmonary infection, we demonstrate that myriocin intratrachea administration, via drug loaded nanocarriers, is able to reduce lung fungal invasion and its derived inflammation.

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