

Pharmacological Modulators of Sphingolipid Metabolism for the Treatment of Cystic Fibrosis Lung Inflammation

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1. Introduction

Cystic Fibrosis (CF) lung disease is characterised by progressive chronic infection and inflammation of the airways. This prolonged airway inflammatory response leads to irreversible lung damage and fibrosis which is believed to be driven by two distinct, coordinated events: *a*) a defective cystic fibrosis transmembrane regulator (CFTR) causes airway surface dehydration and increased mucus viscosity leading to chronic colonization with *Pseudomonas aeruginosa* (*P.aeruginosa*) (Boucher, 2007); *b*) mutated CFTR triggers the generation of pro-inflammatory and chemotactic cytokines orchestrated by bronchial epithelial cells, independently of infection (Rubin, 2007; Elizur et al., 2008). The chemokine IL-8, abundantly expressed at sites of chronic inflammation, seems to play a major role in driving the formation of neutrophil (PMN)-rich exudates into the lung of CF patients (Khan et al., 1995; Noah et al., 1997; DiMango et al., 1998; Puchelle et al., 2001; Joseph et al., 2005; Perez et al., 2007). Therefore, reduction of the exaggerated production of IL-8 is key therapeutic target in CF. Anti-inflammatory drugs are an attractive therapeutic tool in CF aimed to decrease the rate of decline in lung function. However, the inherent complexity of the inflammatory response combined with the obvious dependency on this response to contain infection and the side effect profiles of common anti-inflammatories, have made identifying the most suitable therapy a major priority.

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Consensus is growing on sphingolipids (SLs) as novel targets to cure pulmonary disorders including CF, since modulation of cellular ceramide reduces lung inflammation (Lahiri and Futerman, 2007; Uhlig and Gulbins, 2008). The results in the area of ceramide and CF pathophysiology are very interesting, although contradicting due to the animal models used and methods of ceramide detection (Wojewodka, 2011). The accumulation of ceramide has been identified as one of the key regulators of inflammation in CF airways in different CFTR^{-/-} mouse models (Teichgraber, 2008). On the contrary, decreased ceramide levels have been shown in CFTR ko mice (Guibault, 2008). The possible explanation for this discrepancy seems to be the special diet required for CFTR ko mice, that severely affects the concentration of SLs. Other possible causes, such as genetic determinants, could influence individual levels of SLs (Hicks, 2009). In a different study, no significant difference has been found in basal ceramide levels in immortalised CF bronchial epithelial cells and lung homogenate from CFTR ko mice compared to wild type cells and mice (Yu, 2009). Very importantly, ceramide has been demonstrated to accumulate in the lower airways of CF patients and to be positively associated with neutrophilic inflammation (Brodlie, 2010), supporting the hypothesis that reduction of ceramide may be a therapeutic target for CF lung inflammation.

Extending our previous study (Dehecchi, 2008), we have recently demonstrated that the iminosugar *N*-butyldeoxynojirimycin (miglustat), an inhibitor of the first step in glycosphingolipid (GSL) biosynthesis, reducing the *P.aeruginosa* induced immunoreactive ceramide expression, produces an anti-inflammatory effect in human bronchial epithelial cells *in vitro* and down-regulates the neutrophil chemotaxis in murine lungs *in vivo* (Dehecchi, 2011). These findings strengthen the notion that the metabolism of SLs can be manipulated as a therapeutic option for CF lung disease. With regard to new treatments for CF lung pathology, miglustat deserves great attention since it restores CFTR function in respiratory and pancreatic cells *in vitro* (Norez, 2006; Dehecchi, 2008) and in CF mice (Lubamba, 2009) and produces an anti-inflammatory effect *in vitro* and *in vivo* (Dehecchi, 2011). Notably, miglustat is a FDA-approved and EMA-designated orally bioavailable orphan drug, used in Europe and USA for the treatment of Gaucher disease and other GSL storage diseases.

In this chapter we review the pre-clinical evidence on the anti-inflammatory effect of miglustat in comparative effectiveness studies with the SL inhibitor amitriptyline and the glucocorticoid (GC) dexamethasone. Importance will be placed on the efficacy of each anti-inflammatory molecule to balance between the anti-inflammatory activity and possible impairment of the host defence.

2. CF bronchial cells seem to be resistant to the treatment with glucocorticoids

Chronic inflammation is commonly treated by a number of approaches including fast-acting symptomatic drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids and slow-acting disease-modifying anti-rheumatic drugs, such as low-dose methotrexate. In the treatment of chronic lung inflammation of CF patients, corticosteroids and NSAIDs have garnered the most attention, to date. Although traditional treatments with corticosteroids and ibuprofen have demonstrated potential benefits in CF patients, their use is limited by severe adverse effects, as for high doses of prednisone, or by a narrow pharmacological window, as

in the case of ibuprofen (Birke, 2001; Koehler, 2004; Konstan, 2005). The endobronchial location makes CF pulmonary inflammation potentially amenable to inhaled therapies, thus achieving much higher concentrations in the airway epithelium and limiting the adverse effects of long term systemic use. As the airway epithelium is targeted by inhaled agents, bronchial epithelial cells *in vitro* have been widely used to prove the efficacy of these drugs. The glucocorticoid dexamethasone, largely used in the treatments of inflammatory conditions is scarcely effective in reducing the expression of the chemokine IL-8 in CF bronchial epithelial cells (Figure 1). As a matter of fact, it produces inhibitory effect only at the higher dose (30 μM) (Figure1A) in CF bronchial epithelial IB3-1 cells whereas it fails to reduce the transcription of IL-8 in Cufi-1 cells (Figure 1B). Different from the results obtained in CF CuFi-1 cells, treatment with dexamethasone results in reducing the inflammatory response, in non CF NuLi-1 cells (Figure 1C). These findings suggest that CF bronchial cells seem to be resistant to GC treatment, consistent with scarce efficacy of GC treatment observed in CF patients.

3. *P.aeruginosa* stimulated IL-8 mRNA expression is reduced in CF bronchial epithelial cells treated with inhibitors of SL metabolism miglustat and NB-DGJ

The role of CFTR deficiency in promoting inflammation remains unclear. Inhibition of function of wild type CFTR by CFTR^{inh172} (Perez, 2007) or correction of F508del mutated CFTR function by MPB-07 or miglustat (Dehecchi, 2007; 2008) regulates the inflammatory response to *P. aeruginosa*, suggesting that the pro-inflammatory circuitry in CF airways could be initiated from those epithelial cells lacking CFTR function. However the galactose analogue *N*-butyldeoxygalactonojirimycin (NB-DGJ), which is not a corrector of F508del-CFTR function (Norez, 2006), similarly reduces the PAO1 stimulated IL-8 mRNA expression in CF cells (Figure 2). Additionally this reduction has been obtained both in CF and non CF cells with both miglustat and NB-DGJ (Dehecchi, 2008). Therefore, miglustat affects the inflammatory response to *P. aeruginosa* through a mechanism which, at least partly, is independent of the correction of F508del-CFTR function. As far as the effect of miglustat and NB-DGJ on immune response is concerned, they could affect the host response to *P.aeruginosa* through the regulation of SL metabolism, in particular CerGlcT and/or non lysosomal glucosylceramidase, an activity shared by both compounds (Butters, 2005), thus strengthening the notion that the metabolism of SLs can be manipulated as a therapeutic option for CF lung disease.

4. Amitriptyline reduces IL-8 gene expression in CF bronchial cells

Two main routes have been defined for the generation of ceramide: hydrolysis of sphingomyelin (SM) by acid sphingomyelinase (ASM) and *de novo* biosynthesis (Hannun, 2008). Ceramide generated by ASM plays an important role in the infection by *P. aeruginosa* since it reorganizes into rafts required to internalize bacteria, induce apoptosis and regulate the cytokine response (Grassme', 2003). Treatment of CF mice by ASM inhibitors, such as amitriptyline and others tricyclic antidepressants, has been shown to reduce the degree of inflammation (Teichgraber, 2008; Becker, 2010). Notably, amitriptyline results in dose dependent inhibition of IL-8 transcription by bronchial epithelial cell lines IB3-1 and CuFi-1, infected with *P.aeruginosa* (Figure 3), consistent with the overall anti-inflammatory

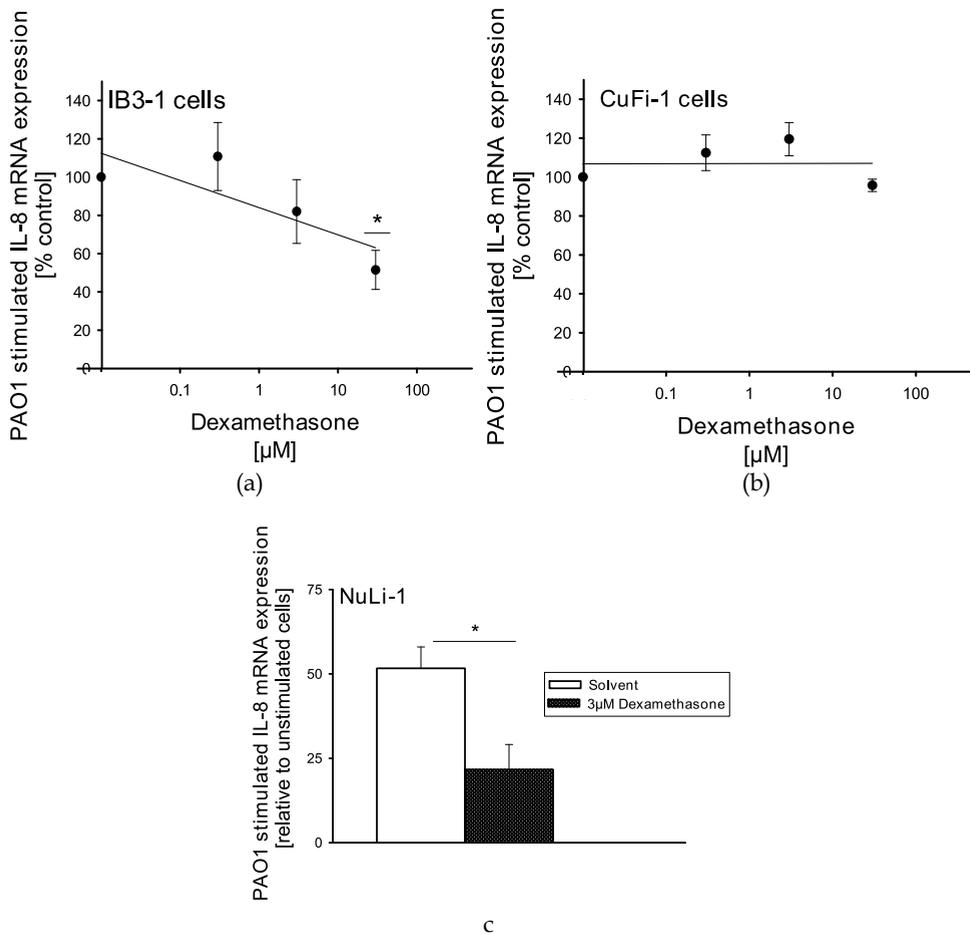


Fig. 1. Effect of dexamethasone on inflammatory response to *P.aeruginosa* in CF and non CF bronchial epithelial cells.

IB3-1 (human bronchial epithelial cell line) (Zeitlin, 1991) (A), CuFi-1 (F508del/F508del CFTR mutant genotype) (B) and NuLi-1 (C), (wild type CFTR) (Zabner, 2003) cell lines were treated with dexamethasone, at doses indicated in the figure, or solvent alone, for 24 hours and then infected with *P.aeruginosa* strain PAO1 (kindly provided by A. Prince, Columbia University, New York)(50CFU/cell) for 4 hours at 37° C. The inflammatory response was evaluated by studying the expression of mRNA of IL-8, measured by Real-time qPCR as described (Dehecchi, 2008) and obtained by comparing the ratio IL-8 and the housekeeping gene GAPDH between non infected and infected cells. Results are expressed as mean \pm standard error of the mean (n=5). Comparisons between groups were made by using Student's *t* test. Statistical significance was defined with $p < 0.05$. *, *p* value < 0.05 .

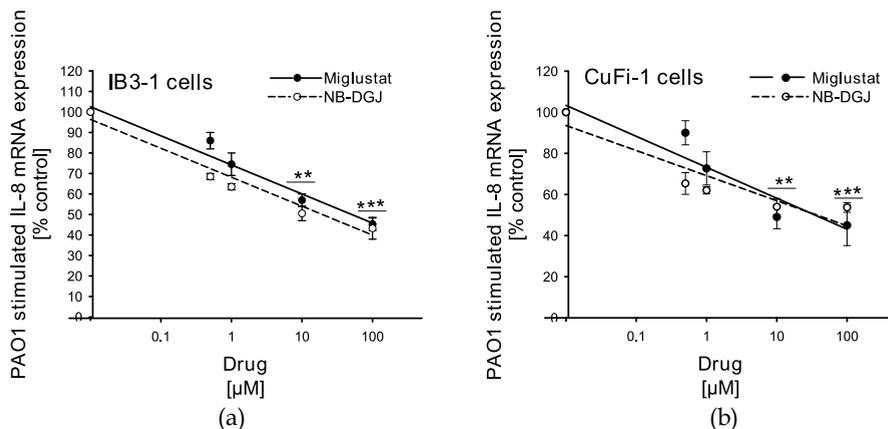


Fig. 2. Result of miglustat and NB-DGJ on *P.aeruginosa* stimulated IL-8 mRNA expression in CF bronchial cell lines IB3-1 and CuFi-1 cells.

IB3-1 (A) and CuFi-1 (B) cells were treated with miglustat, NB-DGJ, at doses indicated in the figure, or solvent alone for 24 hours and then infected with PAO1 as in Figure 1. Stimulated IL-8 mRNA expression was calculated as indicated in the legend of Figure 1 and expressed as % of untreated cells. Data reported are mean ± standard error of the mean of 3 independent experiments, performed in duplicate. **, p value < 0.01; ***, p value < 0.001.

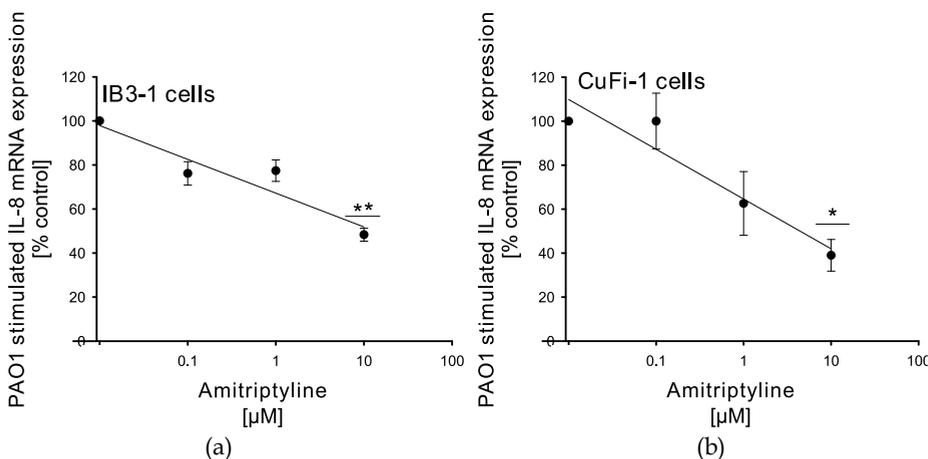


Fig. 3. Result of amitriptyline on *P.aeruginosa* stimulated IL-8 mRNA expression in CF bronchial cell lines IB3-1 and CuFi-1 cells

IB3-1 (A) and CuFi-1 (B) cells were treated with amitriptyline, at doses indicated in the figure, or solvent alone for 24 hours and then infected with PAO1 as in Figure 1. Stimulated IL-8 mRNA expression was calculated as indicated in the legend of Figure 1 and expressed as % of untreated cells. Data reported are mean ± standard error of the mean of 8 (IB3-1) or 6 (CuFi-1) independent experiments, performed in duplicate. *, p value < 0.05; **, p value < 0.01.

effect reported in CF murine lungs (Teichgraber, 2008; Becker, 2010). Interestingly, we have recently demonstrated that, besides inhibiting IL-8 transcription, both miglustat and amitriptyline reduce the *P.aeruginosa* induced immunoreactive ceramide expression (Dehecchi, 2011), by targeting different pathways of SL metabolism: CerGlcT and/or non-lysosomal glucosylceramidase (miglustat) and ASM (amitriptyline). As far as the effect on immune response is concerned, the same overall decrease of ceramide could regulate the transmembrane signaling between the receptors for pathogens and the transcription of the inflammatory genes, thus reducing inflammation.

5. Miglustat and amitriptyline inhibit the pro-inflammatory signaling downstream the receptors for *P.aeruginosa* and for pro-inflammatory cytokines

The importance of ceramide as a pro-inflammatory mediator derives from its capability to activate protein kinases and phosphatases in different downstream pathways and from the generation of second messengers (Hannun, 2008). Ceramide is produced in response to various stimulants such as cytokines, heat, UV radiation, lipopolysaccharide (LPS) and other agents thus leading to specific and overlapping events that include the activation of a common set of kinases and transcription factors. Both miglustat and amitriptyline inhibit the expression of IL-8 mRNA stimulated by either *P.aeruginosa* or TNF α or IL-1 β (Figure 4), indicating that they affect the pro-inflammatory signaling downstream and in common with the receptors for *P. aeruginosa* and for these pro-inflammatory cytokines. Importantly, ceramide-induced activation of the transcription factor NF- κ B and p38 kinase amplifies the production of several inflammatory mediators and adhesion molecules (Won, 2004). Therefore, it can be hypothesized that miglustat and amitriptyline, decreasing plasma membrane ceramide, generated by *P.aeruginosa* infection or pro-inflammatory cytokines, attenuate the activation of NF- κ B mediated signaling cascade which in turn down-regulates the immune response.

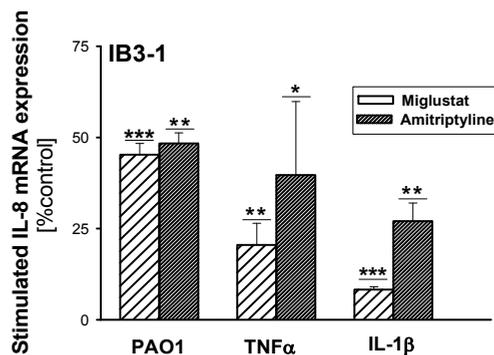


Fig. 4. Effect of miglustat and amitriptyline on IL-8 gene expression stimulated by *P.aeruginosa*, TNF α or IL-1 β .

IB3-1 cells were treated with miglustat (100 μ M), amitriptyline (10 μ M) or solvent alone for 24 hours and then infected with PAO1 or stimulated by the pro-inflammatory cytokines TNF α (10ng/ml) or IL-1 β (50 ng/ml) for 4 hours at 37 $^{\circ}$ C as in Figure 1. Stimulated IL-8 mRNA expression was calculated as indicated in the legend of Figure 1 and expressed as % of

untreated cells. Data reported are mean \pm standard error of the mean of 3 independent experiments performed in duplicate. *, p value < 0.05 ; **, p value < 0.01 ; ***, p value < 0.001 .

6. Miglustat down-regulates the expression of key genes involved in neutrophil chemotaxis in human bronchial CF epithelial cells

The infection of a host by a pathogenic microorganism initiates complex cascade of events directed toward the recruitment of defence mechanisms. Parallel analysis of gene expression provides a new tool for studying interplay of signals and transcriptional responses in biological systems. Infection of IB3-1 cells with the *P.aeruginosa* strain PAO1, for a short time (4 hours), up-regulates the expression of genes involved in the inflammatory response, mainly neutrophil chemotaxis, such as the chemokines IL-8, Gro- $\alpha/\beta/\gamma$, GCP-2, the adhesion molecule ICAM-1, the cytokines IL-1 α/β , IL-6, TNF- α , the antimicrobial peptide HBD-4, Toll-like receptor 2 and NFKB1 (Figure 5A). These results obtained in CF cell line correlate with findings in cultured human bronchial epithelial primary cells derived from the bronchi of CF patients (Figure 5B), which exhibit many of the morphological and functional characteristics believed to be associated with CF airway disease (Neuberger, 2011) and recall many features of the colonization of the respiratory epithelium by pathogens. As far as host defence mechanisms are concerned, bronchial epithelium is not simply a physical barrier against invading pathogens but is also an important source of inflammatory mediators, actively involved in the immune response. Therefore cultured CF bronchial cells provide a useful tool for the pre-clinical testing of novel pharmacotherapies. Indeed, miglustat has an anti-inflammatory effect in CF bronchial cells, since it reduces the expression of key genes induced by *P.aeruginosa* and IL-8 protein release (Dehecchi, 2011). Also amitriptyline has an anti-inflammatory effect in CF bronchial cell lines (Figure 5A) and at lower extent in CF primary cells (Figure 5B). Regarding the therapeutic activity of amitriptyline, it should be noted that systemic inhibition of ASM might negatively affect the host defence, as demonstrated by studies on mice completely lacking ASM which were found to be unable to control infections (Grassme', 2003). Moreover, since amitriptyline inhibits serotonin and noradrenaline uptake in presynaptic nerve ending (Maubach, 1999), it might cause severe adverse effects in long term use in CF children. As with all treatments, utility will largely depend on the balance between potential risks and benefits.

7. Miglustat up-regulates the transcription of the antimicrobial peptide HBD-4

The treatment of CF patients with drug aimed to limit the excessive inflammatory response could mean that they become vulnerable to infections. As a matter of fact, dexamethasone down-regulates the expression of HBD-4 (Figure 6), an antimicrobial peptide induced by infectious or inflammatory stimuli, that plays an important role in the host innate immune response, with strong activity against *P.aeruginosa* (Yanagi, 2005). This result is consistent with suppression of *b*-defensins by GC treatment, already reported (Jang, 2007). On the other hand, well known huge side effects of long term use of steroids limit their clinical utility in CF patients (Nichols, 2008). On the contrary, miglustat up-regulates the expression of HBD-4, both in cell lines and primary cells (Figures 5 and 6), adding ground to the suggestion that miglustat could not compromise the ability to resist infection with pathogens in CF patients. Additionally, results on the safety of miglustat obtained during the first 5 years of the clinical studies, do not report any increased susceptibility to bacterial infections in patients affected by Gaucher disease (Hollak, 2009). All this considered the proximity to a treatment of CF lung inflammation with miglustat is real and promising.

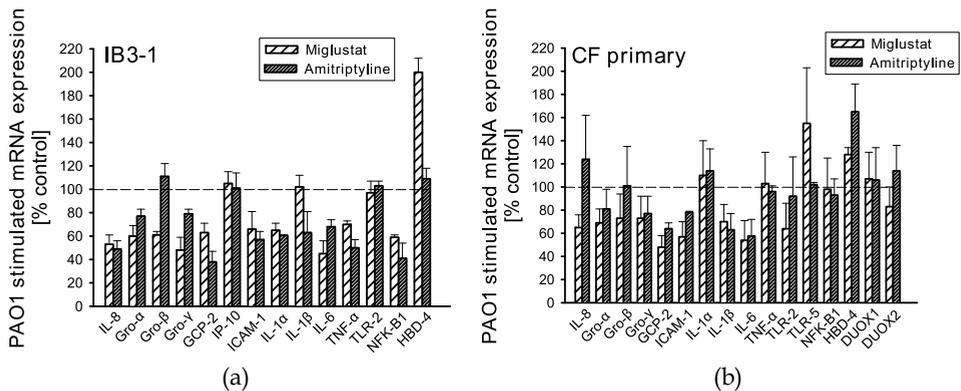


Fig. 5. Inflammatory response to *P.aeruginosa* in IB3-1 and CF primary cells treated with miglustat or amitriptyline.

IB3-1 cells (A) and human airway epithelium reconstituted in vitro with cells isolated from CF patients and cultivated on micro-porous filters at air-liquid interface (MucilAir™, Epithelix Sàrl, Genève, Switzerland) (B) were treated with miglustat (100 μ M), amitriptyline (10 μ M) or solvent alone for 24 hours and then infected with PAO1. The inflammatory response was evaluated by studying the expression of several genes known to be associated with host immune defences by RNA microarrays (TaqMan Low Density Array, Applied Biosystems, Foster City, CA) as detailed (Dehecchi, 2011). Stimulated mRNA expression was calculated as indicated in the legend of Figure 1 and expressed as % of untreated cells. (A) IB3-1 cells. Miglustat significantly reduces the expression of IL-8, ICAM-1, TNF- α (**, p value<0.01), gro- $\alpha/\beta/\gamma$, GCP-2, IL-1 α , IL-6 and NFKB1(*, p value<0.05) and increases the expression of HBD-4 (**, p value<0.01). Amitriptyline significantly reduces the expression of IL-8, Gro- α , ICAM-1 (**, p value<0.01), gro- γ , GCP-2, IL-1 $\alpha/1\beta$, IL-6, TNF- α and NFKB1(*, p value<0.05) ($n=6$). (B) CF primary cells. Miglustat significantly reduces the expression of IL-8, GCP-2, ICAM-1, TNF- α , IL-6 (*, p value<0.05) and increases the expression of HBD-4 (*, p value<0.05). Amitriptyline significantly reduces the expression of GCP-2, ICAM-1, IL-1 β , IL-6 (*, p value<0.05) and increases the expression of HBD-4 (*, p value<0.05) ($n=4$).

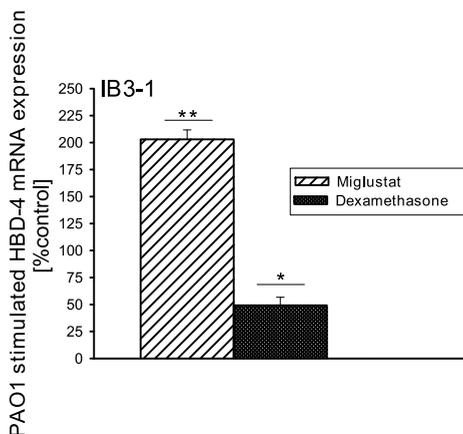


Fig. 6. *P.aeruginosa* stimulated transcription of the antimicrobial peptide HBD-4 in IB3-1 cells: result of miglustat and dexamethasone.

IB3-1 cells were treated with miglustat (100 μ M), dexamethasone (30 μ M) or solvent alone for 24 hours and then infected with PAO1 as in Figure 1. Stimulated HBD-4 mRNA expression was measured by Real-time qPCR as described (Dehecchi, 2011), calculated as indicated in the legend of Figure 1 and expressed as % of untreated cells. Data reported are mean \pm standard error of the mean of 5 independent experiments performed in duplicate. **, p value < 0.01 (miglustat); *, p value < 0.05 (dexamethasone).

8. Miglustat down-regulates neutrophil chemotaxis in the early inflammatory response *in vivo*

The pressing challenge for the discovery of new drugs is to transform findings from bench studies into effective therapies. The success of results obtained with CFTR correctors and potentiators suggests that primary cultures of human airway epithelia will likely be the model system of choice for future proof of principle studies of experimental therapeutics (Neuberger, 2011). With regard to miglustat, the anti-inflammatory effect observed in CF primary cells (Dehecchi, 2011) provides a significant body of evidence concerning the utility of miglustat in modifying lung inflammation. However, an essential requirement for entering clinical trial is a thorough testing in pre-clinical animal models. As a matter of fact development of new animal models of CF such as CFTR knock-out pigs (Rogers, 2008), raises the possibility to employ these animals to evaluate new treatments. Murine models of acute and chronic infection with *P.aeruginosa*, along with mice genetically modified for the CFTR gene, are a key asset in CF research and mimic many of the characteristic features of CF lung pathology (Bragonzi, 2010). Additionally, mice with the airway specific over expression of the Na⁺ channel β ENaC, develop a CF-like lung inflammation (Mall, 2004). These models have provided insights in the effectiveness of anti-inflammatory therapy in reducing lung damage. Interestingly, lung inflammation of β ENaC over expressing mice seems to be resistant to GC treatment (Livraghi, 2009) and amitriptyline reduces the degree of inflammation in CFTR^{-/-} mice (Teichgraber, 2008; Becker 2010). As far as the effectiveness of miglustat is concerned, it reduces the recruitment of PMN into the

bronchoalveolar space induced by intranasal instillation of LPS (Figure 7A) (Dechecchi, 2011). The pharmacokinetic profile and tissue distribution of miglustat, after oral dosing in small rodents, demonstrate that it is well absorbed, exhibits a bioavailability of 40-60%, is widely distributed within the body and, notably, it is present at high concentrations in the lung 1 hour after administration (Treiber, 2007). Indeed, a treatment schedule of an oral administration with miglustat one hour before the intra tracheal inoculum with *P. aeruginosa* is effective in reducing the inflammatory response associated to acute pneumonia in terms of leukocyte recruitment and myeloperoxidase (MPO) activity in the airways (Figure 7B and Figure 7C). Taken together, these results indicate that miglustat may down-regulates neutrophil recruitment in the early phase of the inflammatory response.

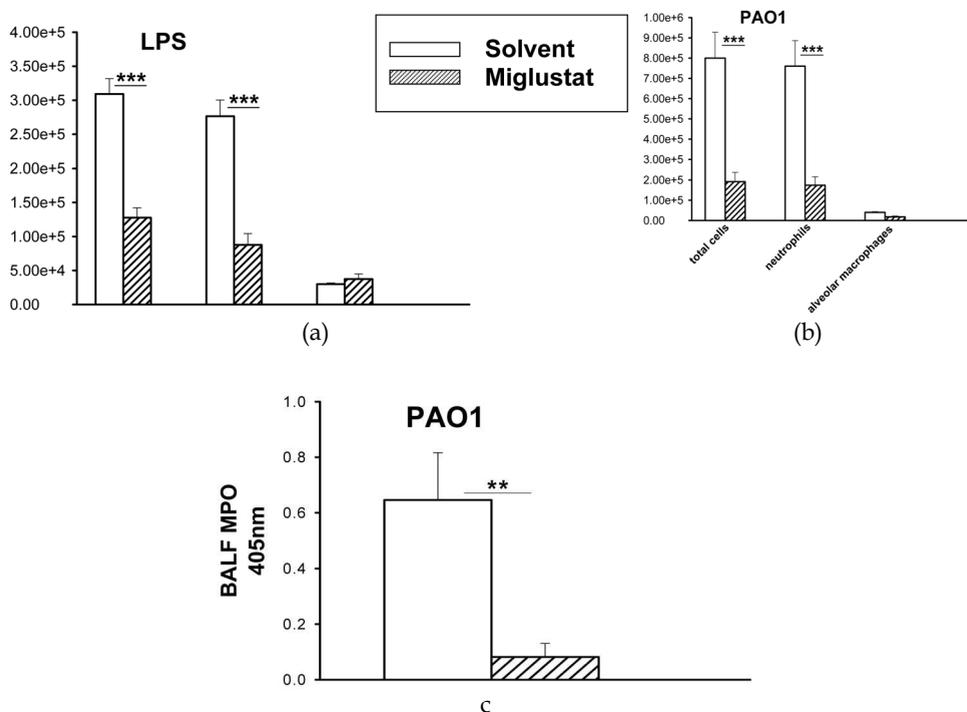


Fig. 7. Effect of miglustat on murine models of lung inflammation.

(A). Aqueous solution of miglustat (100 mg/Kg) or vehicle alone was administered once daily in wild type C57BL/6J mice by intraesophageal gavage, for a period of three consecutive days before pro-inflammatory challenge with LPS by intranasal instillation. Bronchoalveolar lavage fluid (BALF) was examined 4 hrs after challenge, as described (Dechecchi, 2011). Data reported are mean \pm standard error of the mean. $n=6$ (vehicle), $n=12$ (miglustat). ***, p value < 0.001. (B and C). C57BL/6 mice were infected by intra-tracheal injection with the reference *P. aeruginosa* strain PAO1 (1×10^5 CFU) one hour after oral injection with miglustat (400mg/kg), or vehicle. Mice were sacrificed 4 hours after PAO1 injection and the inflammatory response associated to PAO1-induced acute pneumonia in terms of leukocyte recruitment (B) and MPO activity (C) in the airways was analyzed as described (Bragonzi, 2009, Moalli, 2011). Data reported are mean

± standard error of the mean. n=5 (vehicle), n=6 (miglustat). ***, p value < 0.001(leukocyte recruitment); **, p value < 0.01(MPO activity).

9. Conclusions

Strategies aimed to limit the excessive inflammatory response by targeting neutrophil recruitment are a relevant approach for CF patients. In general, each anti-inflammatory molecule should balance between the anti-infective role of neutrophils and the detrimental effects that they produce in the course of chronic inflammation due to the release of proteases and reactive oxygen species. In this respect, while others anti-inflammatory based therapies have failed in humans with CF in the past, the regulation of SLs may represent a useful potential target for pharmacotherapy. This review summarizes evidence derived from the validation of the anti-inflammatory properties of miglustat in bronchial epithelial cells *in vitro* and in murine models of lung inflammation and infection *in vivo*, demonstrating a down-regulation of neutrophils chemotactic signaling. Recalling that miglustat is an orally bioavailable FDA-approved and EMA-designated orphan available drug, therapeutic trials for CF patients could be envisioned in the near future.

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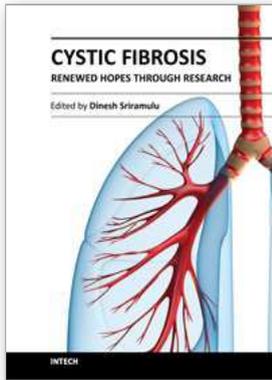
11. References

- Becker K.A.; Riethmüller J.; Lüth A.; Döring G.; Kleuser B.; Gulbins E. (2010). Acid Sphingomyelinase Inhibitors Normalize Pulmonary Ceramide and Inflammation in Cystic Fibrosis. *Am J Respir Cell Mol Biol.* 42(6):716-24
- Birke F.W.; Meade C.J.; Anderskewitz R.; Speck G.A.; Jennewein H.M. (2001). In vitro and in vivo pharmacological Characterization of BIIL 284, a novel and potent leukotriene B(4) receptor antagonist. *J Pharmacol Exp Ther.* 297:458-66
- Boucher R.C. (2007). Evidence for airway surface dehydration as the initiating event in CF airway disease. *J Intern Med* 261:5-16
- Bragonzi A.; Worlitzsch D.; Pier G.B.; Timpert P.; Ulrich M.; Hentzer M.; Andersen J.B.; Givskov M.; Conese M.; Doring G. (2005). Nonmucoïd *Pseudomonas aeruginosa* expresses alginate in the lungs of patients with cystic fibrosis and in a mouse model. *J Infect Dis* 192:410-9
- Bragonzi A.; Paroni M.; Nonis A.; Cramer N.; Montanari S.; Rejman J.; Di Serio C.; Döring G.; Tümmler B. (2009). *Pseudomonas aeruginosa* microevolution during cystic fibrosis lung infection establishes clones with adapted Virulence. *Am J Respir Crit Care Med.* 180(2):138-45.

- Bragonzi A. (2010). Murine models of acute and chronic lung infection with cystic fibrosis pathogens. *Int J Med Microbiol.* 300(8):584-93.
- Brodie M.; McKean M.C.; Johnson G.E.; Gray J.; Fisher A.J.; Corris P.A.; Lordan J.L.; Ward C. (2010). Ceramide is Increased in the Lower Airway Epithelium of People with Advanced Cystic Fibrosis Lung Disease. *Am J Respir Crit Care Med* 182 (3): 369-75
- Butters T.D.; Dwek R.A. and Platt F.M. (2005). Imino sugar inhibitors for treating the lysosomal Glycosphingolipidoses. *Glycobiology* 15, 43R-52R.
- Dehecchi M.C.; Nicolis E.; Bezzerri V.; Vella A.; Colombatti M.; Assael B.M.; Mettey Y.; Borgatti M.; Mancini I.; Gambari R.; Becq F. and Cabrini G. (2007). MPB-07 reduces the inflammatory response to *Pseudomonas aeruginosa* in cystic fibrosis bronchial cells. *Am J Respir Cell Mol Biol* 36, 615-624
- Dehecchi M.C.; Nicolis E.; Norez C.; Bezzerri V.; Borgatti M.; Mancini I.; Rizzotti P.; Ribeiro C.M.; Gambari R.; Becq F.; Cabrini G. (2008). Anti-inflammatory effect of miglustat in bronchial epithelial cells. *J Cyst Fibros.* 7(6):555-65
- Dehecchi M.C.; Nicolis E.; Mazzi P.; Cioffi F.; Bezzerri V.; Lampronti I.; Huang S.; Wiszniewski L.; Gambari R.; Scupoli M.T.; Berton G.; Cabrini G. (2011). Modulators of Sphingolipid Metabolism Reduce Lung Inflammation. *Am J Respir Cell Mol Biol.* Jun 9. [Epub ahead of print]
- DiMango E.; Ratner A.J.; Bryan R.; Tabibi S.; Prince A. (1998) Activation of NF- κ B by adherent *Pseudomonas aeruginosa* in normal and cystic fibrosis respiratory epithelial cells. *J Clin Invest* 101:2598-2605
- Elizur A.; Cannon C.L. ; Ferkol T.W. (2008). Airway inflammation in cystic fibrosis. *Chest* 133:489–495
- Grassme' H.; Jendrossek V.; Riehle A.; von Kürthy G.; Berger J.; Schwarz H.; Weller M.; Kolesnick R.; Gulbins E. (2003). Host defense against *Pseudomonas aeruginosa* requires ceramide-rich membrane rafts. *Nature Medicine* 9:322-330
- Guilbault C.; De Sanctis J.B.; Wojewodka G.; Saeed Z.; Lachance C.; Skinner T.A.; Vilela R.M.; Kubow S.; Lands L.C.; Hajduch M.; Matouk E.; Radzioch D. (2008). Fenretinide corrects newly found ceramide deficiency in cystic fibrosis. *Am J Respir Cell Mol Biol.* 38(1):47-56
- Hannun YA. and Obeid L.M. (2008). Principles of bioactive lipid signaling: lessons from sphingolipids. *Nat Rev Mol Cell Biol* 9:139-150
- Hicks A.A.; Pramstaller P.P.; Johansson A.; Vitart V.; Rudan I.; Ugocsai P.; Aulchenko Y.; Franklin C.; Liebisch G.; Erdmann J.; Jonasson I.; Zorkoltseva I.V.; Pattaro C.; Hayward C.; Isaacs A.; Hengstenberg C.; Campbell S.; Gnewuch C.; Janssens A.C.; Kirichenko A.V.; König I.R.; Marroni F.; Polasek O.; Demirkan A.; Kolcic I.; Schwenbacher C.; Igl W.; Biloglav Z.; Wittman J.C.; Pichler I.; Zaboli G.; Axenovich T.I.; Peters A.; Schreiber S.; Wichmann H.E.; Schunkert H.; Hastie N.; Oostra B.A.; Wild S.H.; Meitinger T.; Gyllenstein U.; van Duijn C.M.; Wilson J.F.; Wright A.; Schmitz G.; Campbell H.; (2009). Genetic determinants of circulating sphingolipid concentrations in European populations. *PLoS Genet.* 5:1-11
- Hollak C.E.; Hughes D.; van Schaik I.N.; Schwierin B.; Bembi B. (2009). Miglustat (Zavesca) in type 1 Gaucher disease: 5-year results of a post-authorization safety surveillance programme. *Pharmacoepidemiol Drug Saf.* 18(9):770-7
- Jang B.-C.; Lim K.-J.; Suh M.-H.; Park J.-G. and Suh S. (2007). Dexamethasone suppresses interleukin-1 β -induced human β -defensin 2 mRNA expression: involvement of p38

- MAPk, JNK, MKP-1 and NF- κ B transcriptional factor in A549 cells. *FEMS Immunol Med Microbiol* 51: 171-184
- Joseph T.; Look D.; Ferkol T. (2005). NF- κ B activation and sustained IL-8 gene expression in primary cultures of cystic fibrosis airway epithelial cells stimulated with *Pseudomonas aeruginosa*. *Am J Physiol Lung Cell Mol Physiol* 288: L471-L479
- Khan T.Z. ; Wagener J.S. ; Bost T. ; Martinez J. ; Accurso F.J. ; Riches D.W. ; (1995). Early pulmonary inflammation in infants with cystic fibrosis. *Am J Respir Crit Care Med* 151:1075-1082
- Koehler D.R.; Downey G.P.; Sweezey N.B.; Tanswell A.K.; Hu J. (2004) Lung inflammation as a therapeutic target in cystic fibrosis. *Am J Respir Cell Mol Biol* 31:377-381
- Konstan M.W.; Doring G. ;Lands L.C.; Hilliard K.A.; Koker P.; Bhattacharya. ; Staab A.; Hamilton A.L. (2005). Results of a phase II clinical trial of BIIL284 BS for the treatment of CF lung disease. *Pediatric Pulmonol S* 28: 125-126
- Lahiri S. and Futerman A.H. (2007).The metabolism and function of sphingolipids and glycosphingolipid. *Cell Mol Life Sci.* 64: 2270-2284.
- Livraghi A.; Grubb B.R.; Hudson E.J.; Wilkinson K.J.; Sheehan J.K.; Mall M.A. O'Neal W.K. Boucher R.C.; Randell S.H. (2009). Airway and lung pathology due to mucosal surface dehydration in {beta}-epithelial Na⁺ channel- overexpressing mice: role of TNF- α and IL-4R α signaling, influence of neonatal development, and limited efficacy of glucocorticoid treatment.*J Immunol.* 182(7):4357-67
- Lubamba B.; Lebacqz J.; Lebecque P.; Vanbever R.; Leonard A.; Wallemacq P.; Leal T. (2009). Airway delivery of low-dose miglustat normalizes nasal potential difference in F508del cystic fibrosis mice. *Am J Respir Crit Care Med.* 179:1022-8.
- Mall M.; Grubb B.R.; Harkema J.R.; O'Neal W.K.; Boucher R.C. (2004). Increased airway epithelial Na⁺ absorption produces cystic fibrosis-like lung disease in mice. *Nat Med* 10:487-493
- Maubach K.A.; Rupniak N.M.; Kramer M.S.; Hill R.G. (1999). Novel strategies for pharmacotherapy of depression. *Curr Opin Chem Biol.* 481-8
- Moalli F, Paroni M, Véliz Rodriguez T, Riva F, Polentarutti N, Bottazzi B, Valentino S, Mantero S, Nebuloni M, Mantovani A, Bragonzi A, Garlanda C. The therapeutic potential of the humoral pattern recognition molecule PTX3 in chronic lung infection caused by *Pseudomonas aeruginosa*. *J Immunol.* 2011 May 1;186(9):5425-34.
- Neuberger T.; Burton B.; Clark H. and Van Goor F. (2011). Use of primary cultures of human bronchial epithelial cells Isolated from cystic fibrosis patients for the pre-clinical testing of CFTR modulators, In: *Cystic Fibrosis, Methods in Molecular Biology*, M.D. Amaral and K. Kunzelmann (eds), 39-54, Springer Science
- Nichols D.P.; Konstan M.W. and Chmiel J.F. (2008) Anti-inflammatory therapies for cystic fibrosis-related lung disease. *Clinic Rev Aller Immunol* 35: 135-153
- Noah T.L.; Black H.R.; Cheng P.W.; Wood R.E.; Leigh M.W. (1997) Nasal and bronchoalveolar lavage fluid cytokines in early cystic fibrosis. *J Infect Dis* 175: 638-647
- Norez C.; Noel S.; Wilke M.; Bijvelds M.; Jorna H.; Melin P.; DeJonge H. and Becq F. (2006). Rescue of functional Δ F508- CFTR channels in cystic fibrosis epithelial cells by the α -glucosidase inhibitor miglustat. *FEBS Lett.* 580:2081- 2086.

- Perez A.; Issler A.C.; Cotton C.U.; Kelley T.J.; Verkman A.S.; Davis P.B. (2007). CFTR inhibition mimics the cystic fibrosis inflammatory profile. *Am J Physiol Lung Cell Mol Physiol* 292:L383–395
- Puchelle E.; De Bentzmann S.; Hubeau C.; Jacquot J.; Gaillard D. (2001). Mechanisms involved in cystic fibrosis airway inflammation. *Pediatr Pulmonol* S23:143-5
- Rogers C.S.; Stoltz D.a.; Meyerholz D.K. et al (2008). Disruption of the CFTR gene produces a model of cystic fibrosis in newborn pigs. *Science* 321: 1837-41
- Rubin BK. (2007). CFTR is a modulator of airway inflammation. *Am J Physiol Lung Cell Mol Physiol* 292:L381–382.
- Teichgraber V.; Ulrich M.; Endlich N.; Riethmüller J.; Wilker B.; De Oliveira-Munding C.C.; van Heeckeren A.M.; Barr M.L.; von Kürthy G.; Schmid K.W.; Weller M.; Tümmler B.; Lang F.; Grassme H.; Döring G.; Gulbins E (2008). Ceramide accumulation mediates inflammation, cell death and infection susceptibility in cystic fibrosis. *Nature Med* 14:382-391
- Treiber A.; Morand O. and Clozel M. (2007) The pharmacokinetics and tissue distribution of the glucosylceramide synthase inhibitor miglustat in the rat. *Xenobiotica* 37:298-314.
- Uhlig S.; Gulbins E. (2008). Sphingolipids in the lungs. *Am J Respir Crit Care Med*. 178(11):1100-14
- Vicentini L.P.; Mazzi L.; Caveggon E.; Continolo S.; Fumagalli L.; Lapinet-Vera J.A.; Lowell C.A.; Berton G. (2002). FcγR deficiency results in defective eosinophil recruitment to the lung during allergic airway inflammation. *J Immunol* 168: 6446
- Wojewodka G.; De Sanctis J.B. and Radzioch D. (2011). Ceramide in Cystic Fibrosis : A potential new target for therapeutic intervention. *J of Lipids* 2011: 1-13
- Won J.S.; Im Y.B.; Khan M.; Singh A.K.; Singh I. (2004). The role of neutral sphingomyelinase produced ceramide in lipopolysaccharide-mediated expression of inducible nitric oxide synthase. *J Neurochem*. 88:583-93
- Yanagi S.; Ashitani J.; Ishimoto H.; Date Y.; Mukae H.; Chino N.; Nakazato M. (2005). Isolation of human beta-defensin-4 in lung tissue and its increase in lower respiratory tract infection. *Respir Res*. 6:130
- Yu H.; Zeidan Y.H.; Wu B.X.; Jenkins R.W.; Flotte T.R.; Hannun Y.A.; Virella-Lowell I. (2009). Defective acid sphingomyelinase pathway with *Pseudomonas aeruginosa* infection in cystic fibrosis. *American Journal of Respiratory Cell and Molecular Biology* 41:367-375
- Zabner J.; Karp P.; Seiler M.; Phillips S.L. Mitchell C.J.; Saavedra M.; Welsh M.; Klingelutz A.J. (2003). Development of cystic fibrosis and non cystic fibrosis airway cell lines. *Am. J. Physiol. Lung Cell Mol. Physiol.* 284:L844-L854.
- Zeitlin P.L.; Lu L.; Rhim J.; Cutting G.; Stetten G.; Kieffer K.A.; Craig R.; Guggino W.B. (1991). A cystic fibrosis bronchial epithelial cell line: immortalization by adeno-12-SV40 infection. *Am. J. Respir. Cell Mol Biol* 4: 313-319.



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Living healthy is all one wants, but the genetics behind creation of every human is different. As a curse or human agony, some are born with congenital defects in their menu of the genome. Just one has to live with that! The complexity of cystic fibrosis condition, which is rather a slow-killer, affects various organ systems of the human body complicating further with secondary infections. That's what makes the disease so puzzling for which scientists around the world are trying to understand better and to find a cure. Though they narrowed down to a single target gene, the tentacles of the disease reach many unknown corners of the human body. Decades of scientific research in the field of chronic illnesses like this one surely increased the level of life expectancy. This book is the compilation of interesting chapters contributed by eminent interdisciplinary scientists around the world trying to make the life of cystic fibrosis patients better.

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