CAT-5571 IMPROVES THE CLEARANCE OF INTRACELLULAR *BURKHOLDERIA CENOCEPACIA* FROM PRIMARY CYSTIC FIBROSIS$^{F508del/F508del}$ MACROPHAGES

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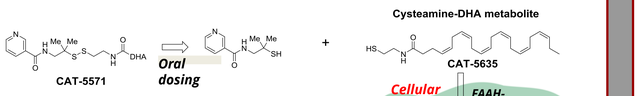
INTRODUCTION

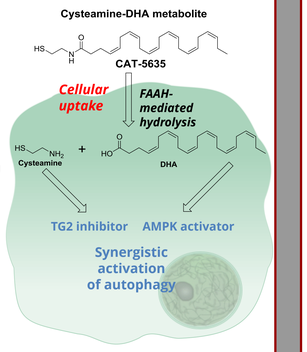
The opportunistic pathogen *Burkholderia cenocepacia* causes severe infections with probable fatal outcome in patients with cystic fibrosis (CF) because it harbors a natural resistance to most antibiotics and therefore it is hard to eradicate from colonized lung tissue. In healthy Non-CF macrophages intracellular *B. cenocepacia* is typically restricted via autophagy. However, autophagic activity is compromised in CF$^{F508del/F508del}$ macrophages resulting in delayed maturation and acidification of the *B. cenocepacia* phagosome, leading to survival of the organism within vacuolar compartments (Abdulrahman et al. Autophagy 2011;7:11,1359-1370). A novel strategy to restore autophagy in CF is the molecular conjugate CAT-5571 known to potently activate autophagy in human primary CF$^{F508del/F508del}$ bronchial epithelial (hBE) cells. The biologically active component of CAT-5571 consists of a cysteamine moiety that is covalently linked to the omega-3 fatty acid docosahexaenoic acid (DHA) (Vu et al. J. Med. Chem. 2017;60,458−473). Cysteamine alone was shown to improve *P. aeruginosa* clearance from CF$^{F508del/F508del}$ macrophages, yet at very high concentrations (250µM) (Ferrari et al. Cell Death Dis. 2017;8(1):e2544). In contrast, the molecular conjugate CAT-5571 activated autophagy in hBE cells at concentrations below 1µM leading to reduced *P. aeruginosa* survival. Furthermore, in CF$^{F508del/F508del}$ mutant mice, treatment with the orally bioavailable conjugate CAT-5571 restored the depressed autophagy markers Beclin-1 and LC3-II.

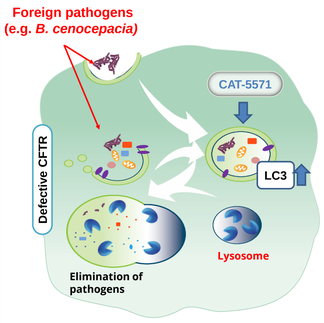
To examine if CAT-5571 also promotes *B. cenocepacia* clearance, we infected murine CF$^{F508del/F508del}$ macrophages treated with and without CAT-5571 and examined intracellular bacterial growth. Survival of *B. cenocepacia* in CF$^{F508del/F508del}$ macrophages was significantly reduced after treatment with CAT-5571. This was not due to a direct bactericidal effect of CAT-5571 on *B. cenocepacia*, since no difference in growth could be observed in LB media with or without the addition of CAT-5571. To further analyze if CF$^{F508del/F508del}$ macrophages exhibit increased autophagic activity after stimulation with CAT-5571, we evaluated the expression of Beclin1 and LC3-II via Western Blot analysis revealing the restoration of these autophagy markers upon CAT-5571 treatment. Thus, CAT-5571 has the potential to re-establish functional autophagy to enhance bacterial clearance in CF. Therefore, CAT-5571 potentially could serve as a new treatment to prevent or eliminate chronic antibiotic-resistant infections in the lungs of CF patients.



Independent but intersecting pathways to modulate autophagy

Cysteamine inhibits transglutaminase 2 (TG2)
DHA (an omega-3 fatty acid) activates AMPK



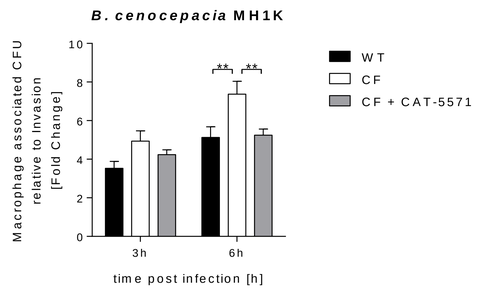


HYPOTHESIS

CAT-5571 delivers *B. cenocepacia* to lysosomes for degradation through autophagy in murine CF$^{F508del/F508del}$ macrophages

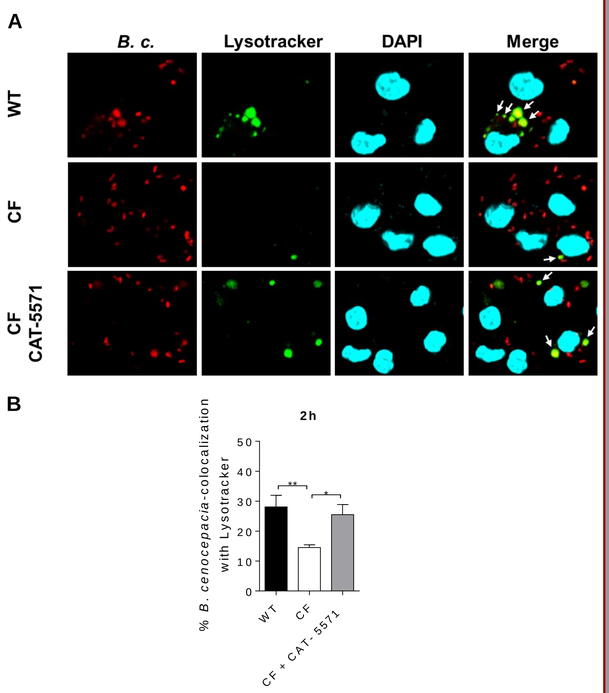
RESULTS

CAT-5571 restores bacterial clearance in CF$^{F508del/F508del}$ macrophages



Intracellular CFU of *B. cenocepacia* MH1K in WT and CF$^{F508del/F508del}$ macrophages. Macrophages were infected with an MOI of 10:1. Values are means and standard error of the mean (SEM) of seven independent experiments. Statistical analyses were performed using Two-way ANOVA (** p< 0.01).

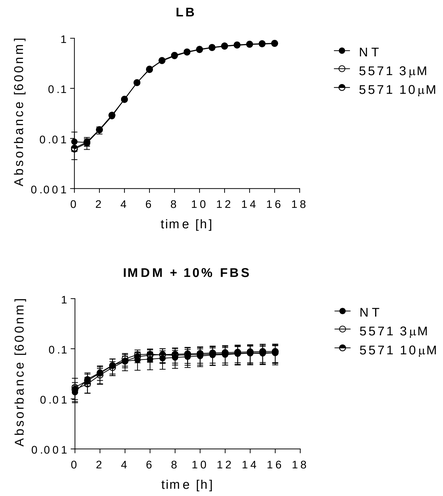
CAT-5571 restores the co-localization of *B. cenocepacia* with lysosomes in CF$^{F508del/F508del}$ macrophages



CAT-5571 promotes trafficking of *B. cenocepacia* to lysosomes in CF$^{F508del/F508del}$ macrophages. (A) Immunofluorescence assay of *B. cenocepacia*-infected macrophages derived from WT and CF$^{F508del/F508del}$ mice at 2 hours post infection. . **(B)** Quantification of *B. cenocepacia*/Lysotracker co-localization. Images were taken with Fluoview10i confocal microscope. Values are mean and standard error of the mean (SEM) of four independent experiments (n=4). Statistical analyses was performed using One-way ANOVA (* p < 0.05, ** p < 0.01).

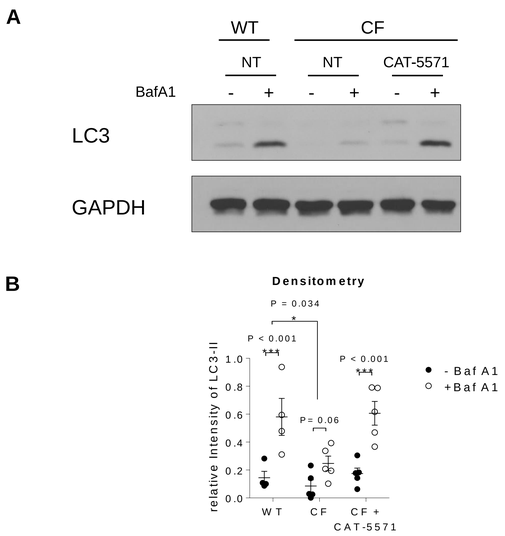
RESULTS

CAT-5571 does not directly affect the growth of *B. cenocepacia*



***B. cenocepacia* MH1K Growth Curves in LB and IMDM media.** *B. cenocepacia* was inoculated at OD_{600} = 0.01 (LB) or OD_{600} = 0.05 (IMDM) and grown for 16h. Values are means and standard error of the mean (SEM) of three independent experiments (n=3). Statistical analysis was performed using repeated measures Two-way ANOVA .

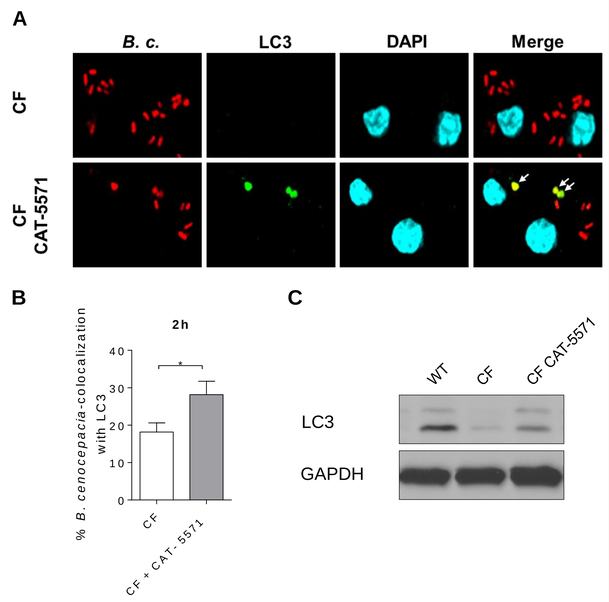
CAT-5571 restores autophagic flux in CF$^{F508del/F508del}$ macrophages



CAT-5571 restores compromised autophagy in CF$^{F508del/F508del}$ macrophages. (A) Western Blot Analysis of macrophages treated with 10µM CAT-5571 and 100nM Bafilomycin A1 (BafA1). **(B)** Densitometry data represented as mean and standard error of the mean (SEM). Statistical analysis was performed using a linear mixed model (*** p < 0.001).

RESULTS

CAT-5571 improves autophagy in *B.* cenocepacia-infected CF$^{F508del/F508del}$ macrophages



CAT-5571 improves the autophagic response against *B. cenocepcia* in CF$^{F508del/F508del}$ macrophages. (A) Immunofluorescence assay of *B. cenocepacia*-infected macrophages derived from WT and CF$^{F508del/F508del}$ mice at 2 hours post infection. Images were taken with Fluoview10i confocal microscope. **(B)** Quantification of *B. cenocepacia*/LC3 co-localization. Values are mean and standard error of the mean (SEM) of three independent experiments (n=3). Statistical analyses was performed using Student *t* Test (* p < 0.05). **(C)** Western Blot analysis of LC3-II conversion in *B. cenocepacia*-infected macrophages at 6 hours post infection.

SUMMARY

- CAT-5571 enhances the clearance of *B. cenocepacia* in CF$^{F508del/F508del}$ macrophages
- CAT-5571 does not directly affect growth of *B. cenocepacia* in LB or IMDM cell culture media
- CAT-5571 restores co-localization of *B. cenocepacia* with Lysotracker green in CF$^{F508del/F508del}$ macrophages
- CAT-5571 restores autophagic flux in unstimulated CF$^{F508del/F508del}$ macrophages
- CAT-5571 increases co-localization of *B. cenocepacia* with LC3 in CF$^{F508del/F508del}$ macrophages
- CAT-5571 increases *B. cenocepacia*-induced LC3-II conversion in CF$^{F508del/F508del}$ macrophages

CONCLUSION

CAT-5571 restores the depressed autophagy in CF macrophages to enhance bacterial clearance. CAT-5571 potentially could serve as a new treatment to prevent or eliminate chronic antibiotic-resistant infections in the lungs of CF patients.

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