CAT-5571 as a Novel Autophagy Activator that Enhances the Clearance of Pseudomonas aeruginosa

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ABSTRACT

In CF patients, the lack of a proper functioning CFTR causes a chronic lung infection that is difficult to treat with conventional antibiotics. Pseudomonas aeruginosa is a common and particularly virulent pathogen that can cause a significant level of morbidity and mortality. Intracellular bacterial colonization of P. aeruginosa in macrophages, mast cells or epithelial cells is particularly difficult to eradicate with antibiotics, even with those that are cell-permeable. Autophagy is a catabolic process that cells use to degrade various defective proteins/foreign pathogens and convert them into useful cellular building blocks such as amino acids and lipids. In CF, it is known that autophagy is impaired and this can further compromise the patient’s ability to clear the chronic lung infection. Autophagy activation enables an alternative mechanism to clear the bacterial infection out of cells; and therefore, could potentially be useful when used in combination with anti-infective agents. The fatty acid cysteamine conjugate CAT-5571 can activate autophagy in cultured primary homozygous F508del human bronchial epithelial (hBE) cells at concentrations as low as 0.3 μM. In an in vitro study involving hBE cells that had been infected with P. aeruginosa, a significant reduction in the intracellular bacterial load was observed when cells were pre-treated with CAT-5571. The bacterial clearance was demonstrated first in an in vivo study using female BALB/c mice that were dosed orally with CAT-5571 for 3.5 days prior to infection with a lethal challenge of P. aeruginosa. CAT-5571 was then evaluated in a chronic P. aeruginosa infection model involving Cftr gut corrected mouse B6.129(Cg-Rt1/JawCwr (gut corrected F508del). In this in vivo model of CF lung infection and inflammation, treatment with CAT-5571 resulted in a decrease in bacterial load (8256 ± 36484 without drug to 1657 ± 1406 with drug, n = 7). Although there was no difference in the total white blood cell count in bronchoalveolar lavage fluid, there was a shift away from neutrophils (26.4 ± 6 vs 8 ± 3, n ≥ 6) and increased number of macrophages (71 ± 29 versus 89 ± 6, n ≥ 6) all parameters p<0.05, using the analysis of variance between vehicle and CAT-5571 treated animals. CAT-5571 represents a potential new therapeutic to treat the chronic lung infection that is commonly present in CF.

MATERIALS & METHODS

Application: Since patients with CF not only have inflammation, but they are also chronically colonized with bacteria, pre-clinical studies were designed to demonstrate the anti-inflammatory potential of CAT-5571 and the overall impact on Pseudomonas aeruginosa colonization. The murine model of CF in which the Cftr gene is either knocked out or dysfunctional can provide a consistent and reproducible model in which to measure the differences in the CF host’s inflammatory response to pathogens relative to controls with functional Cftr providing an ideal window for studying anti-inflammatory drugs in the context of ongoing chronic infection. In CF patients, the lack of a proper functioning CFTR causes a chronic lung infection that is particularly difficult to treat with conventional antibiotics. Pseudomonas aeruginosa is a common and particularly virulent pathogen that can cause a significant level of morbidity and mortality. The fatty acid cysteamine conjugate CAT-5571 can activate autophagy in cultured primary homozygous F508del human bronchial epithelial (hBE) cells at concentrations as low as 0.3 μM. In an in vitro study involving hBE cells that had been infected with P. aeruginosa, a significant reduction in the intracellular bacterial load was observed when cells were pre-treated with CAT-5571. The bacterial clearance was demonstrated first in an in vivo study using female BALB/c mice that were dosed orally with CAT-5571 for 3.5 days prior to infection with a lethal challenge of P. aeruginosa. CAT-5571 was then evaluated in a chronic P. aeruginosa infection model involving Cftr gut corrected mouse B6.129(Cg-Rt1/JawCwr (gut corrected F508del). In this in vivo model of CF lung infection and inflammation, treatment with CAT-5571 resulted in a decrease in bacterial load (8256 ± 36484 without drug to 1657 ± 1406 with drug, n = 7). Although there was no difference in the total white blood cell count in bronchoalveolar lavage fluid, there was a shift away from neutrophils (26.4 ± 6 vs 8 ± 3, n ≥ 6) and increased number of macrophages (71 ± 29 versus 89 ± 6, n ≥ 6) all parameters p<0.05, using the analysis of variance between vehicle and CAT-5571 treated animals. CAT-5571 represents a potential new therapeutic to treat the chronic lung infection that is commonly present in CF.

I. In Vitro and In Vivo Development of CAT-5571

CAT-5571 synergistically activated autophagy in human colon and human primary bronchial epithelial cells

When mice were pre-treated with CAT-5571 for 3.5 days prior to a lethal challenge of P. aeruginosa, there was a significant improvement in clinical score and overall survival.

II. In Vivo Therapeutic Testing of CAT-5571 in the Murine Model of CF Infection and Inflammation

CAT-5571 causes a significant reduction in the intracellular P. aeruginosa in F508del CFHBEs

When mice were pre-treated with CAT-5571 for 3.5 days prior to a lethal challenge of P. aeruginosa, there was a significant improvement in clinical score and overall survival.

CONCLUSIONS

• CAT-5571 activates autophagy in human colon and human primary bronchial epithelial cells.
• CAT-5571 is bioactive in vivo post-administration into mice, rats and dogs. The end-point of autophagy could be measured in all cases.
• The impact of CAT-5571 appears to be through decreasing intracellular colonization of Pseudomonas aeruginosa.
• In the murine model of CF lung infection and inflammation, CAT-5571 decreased bacterial load and lung neutrophils consistent with improved outcomes in the CF murine model.

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