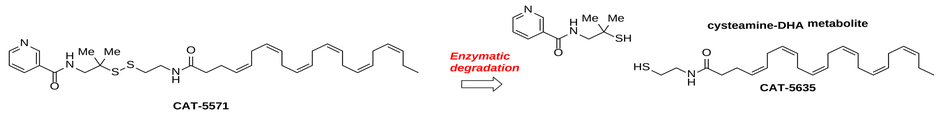
CAT-5571 AS A NOVEL AND POTENT AUTOPHAGY ACTIVATOR THAT ENHANCES THE TRAFFICKING OF THE F508DEL-CFTR

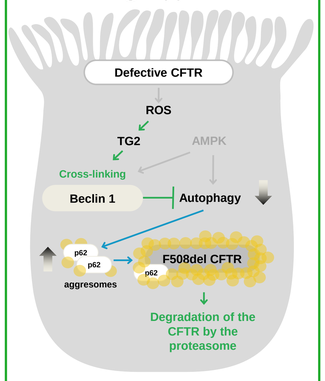
Liu, F.[1]; Carney, D.[1]; Chandran, S.[1]; Lee, D.[1]; Lonkar, P.[1]; Nichols, A.[1]; Picarella, D.[1]; Ting, A.[1]; Webb, S.[1]; Wensley, A.[1]; Yeager, M.[1]; Bridges, R. J.[2], PenaRasgado, C.[2]; Lacerda, A.[3]; Bordwell, C.[3]; Vu, C. B.[1] 1. Catabasis Pharmaceuticals, Cambridge, MA, USA. 2. Chicago Medical School, North Chicago, IL, USA. 3. Charles River, Cleveland, OH, USA.

Introduction

Autophagy is a physiological process that helps maintain cellular homeostasis through the orderly degradation and recycling of dysfunctional cellular components. Depressed autophagy has previously been reported in cystic fibrosis patients with the common F508del mutation. Herein we describe the biological characterization of an autophagy activator, a covalent conjugate between cysteamine and the omega-3 fatty acid docosahexaenoic acid (CAT-5635), in primary homozygous F508del human bronchial epithelial (hBE) cells.

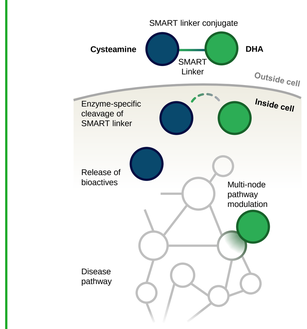


Working hypothesis

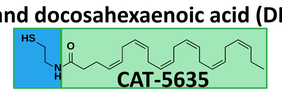


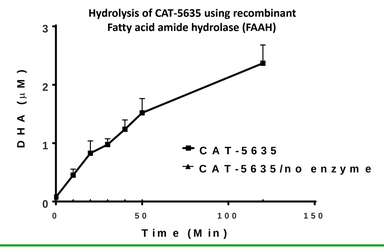
Autophagy activation by the simultaneous inhibition of TG2 and activation of AMPK.

This reduces p62 and allows the trafficking of the CFTR to the cell surface

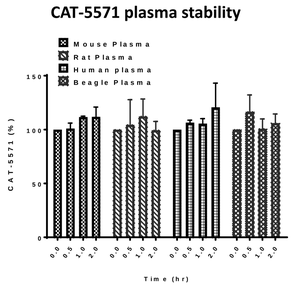


A covalent conjugate of cysteamine and docosahexaenoic acid (DHA)

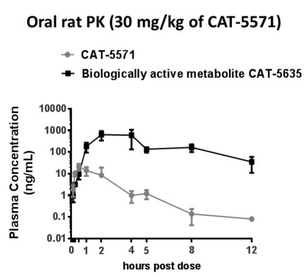




CAT-5571 plasma stability



Oral rat PK (30 mg/kg of CAT-5571)



Methods

TECC-24: Primary homozygous F508del hBE cells were incubated in differentiation media at 37 °C for 24 hr. The media was then switched to Coon's F12, with test compounds added back. Plate was incubated at 37 °C for 4 hr prior to I_{EQ} measurements. Representative I_{EQ} traces following the addition of benzamil (3 µM) to first block currents deriving from ENaC. Forskolin (10 µM) was added and the I_{EQ} was recorded over a 27 min period at 37 °C. Bumetanide (20 µM) or $CFTR_{inh}$-172 (20 µM) was added to block the chloride secretion from the CFTR. Quantification of the bumetanide or CFTRinh-172 -inhibited CFTR chloride current for the indicated treatment groups, ΔI_{EQ} (µA/cm^2). Quantification of the AUC (computed for the time period that spans the I_{EQ} value at the time of forskolin addition up to the I_{EQ} value at the time of bumetanide or CFTRinh-172 addition, min * µA/cm^2).

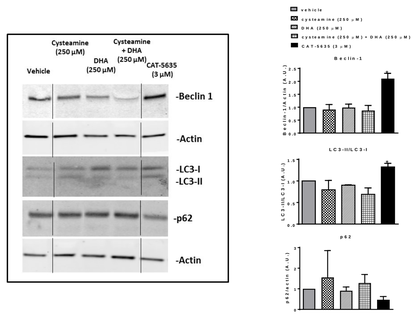
Conclusion

- CAT-5571 delivers a sustained exposure of CAT-5635, a covalent conjugate of cysteamine and DHA
- CAT-5635 potently activates autophagy in cultured primary homozygous F508del hBE cells at ≤ 3 µM. Neither cysteamine nor DHA (alone or in combination) activates autophagy at the higher concentration (250 µM).
- CAT-5635 enhances the correction of the F508del-*CFTR* relative to the VX-809/VX-770 combination
  - Expression of CFTR Band B/C
  - CFTR chloride channel conductance

CAT-5571 represents a potential novel therapeutic approach for the treatment of cystic fibrosis with the F508del mutation

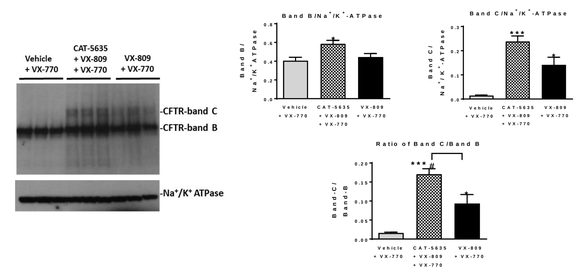
Results

CAT-5635 activates autophagy in primary homozygous F508del hBE monolayers



Primary homozygous F508del hBE cells (Charles River Labs, patient code KKCFFT004I) treated for 24 hrs at 37 °C with the above treatment groups. Quantification of the immunoblots shown. Error bars represent standard error mean (SEM, n = 3). *p < 0.05, compared to the vehicle with ANOVA followed by Dunnett's multiple comparison test.

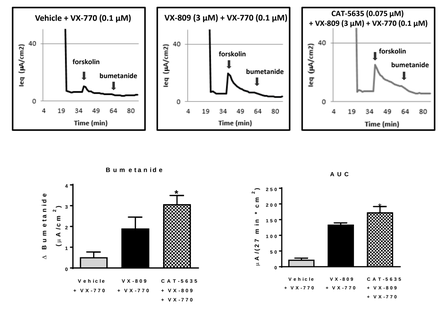
CAT-5635 increased CFTR Band-B and Band-C in the presence of VX-809 and VX-770



Primary homozygous F508del hBE cells (Charles River Labs, patient code KKCFFT006F) treated for 24 hrs with the above treatment groups. Quantification of the immunoblots for the CFTR Band B, the CFTR Band C, and the ratio of Band C to Band B. Error bars represent standard deviation; SEM ± SEM, n = 3, * p < 0.05, ***p < 0.005, compared to Vehicle/VX770; # p < 0.05, compared to the VX-809/VX-770 treatment group, with ANOVA followed by Dunnett's multiple comparison test.

A triple combination consisting of CAT-5635 enhanced the correction of the F508del-*CFTR* relative to the VX-809/VX-770 combination

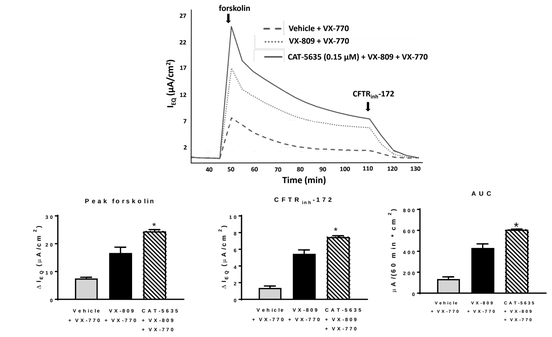
CAT-5635 pre-incubated with VX-809 + VX-770 for 24 hrs at 37 °C



Robert J. Bridges, homozygous F508del hBE, CFFT0018I

(SEM, n = 3). * p < 0.05, compared to VX-809/VX-770 with Dunnett's multiple comparison test.

CAT-5635 pre-incubated with VX-809 + VX-770 for 24 hrs at 37 °C

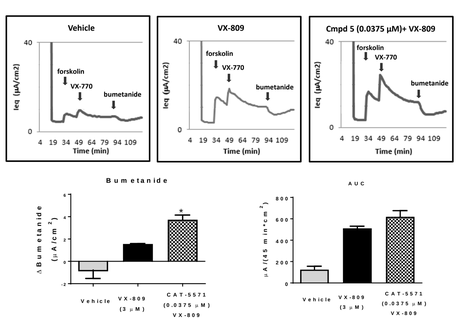


Charles River, homozygous F508del hBE, KKCFFT006F

(SEM, n = 4). * p < 0.05, compared to VX-809/VX-770 with Dunnett's multiple comparison test.

A triple combination consisting of CAT-5571 enhanced the correction of the F508del-*CFTR* relative to the VX-809/VX-770 combination

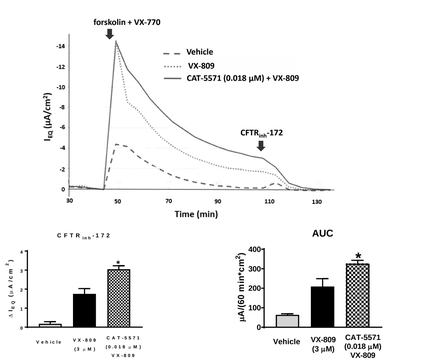
CAT-5571 pre-incubated with VX-809 for 24 hrs at 37 °C



Robert J. Bridges, homozygous F508del hBE, CFFT0028H

(SEM, n = 3). * p < 0.05, compared to VX-809/VX-770 with Dunnett's multiple comparison test.

CAT-5571 pre-incubated with VX-809 for 24 hrs at 37 °C



Charles River, homozygous F508del hBE, KKCFFT0012I

(SEM, n = 4). * p < 0.05, compared to VX-809/VX-770 with Dunnett's multiple comparison test.

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