Determining the relationship between neutrophil elastase and salivary α-amylase in cystic fibrosis sputum and oral samples

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Abstract

Cystic fibrosis (mucoviscidosis) is an autosomal recessive genetic disorder with a poor prognosis and no known cure. Caused by mutations in the gene for cystic fibrosis transmembrane conductance regulator (CFTR), a protein that regulates the viscosity of bodily fluids, cystic fibrosis leads to the buildup of viscid mucus in the lungs that provides pathogens an optimal environment to thrive. As a result, CF patients face frequent infections and must regularly use ineffective general antibiotics. Therefore, microbiome sequencing of CF patients is critical to the development of targeted treatments. However, as samples are taken orally, saliva contamination is an issue and may cause samples to misrepresent sequencing results. To determine the severity of contamination, we conducted assays on sputum and mouth rinse samples of CF patients and calculated the concentrations of two specific enzymes, neutrophil elastase (NE) and salivary α-amylase (SA), that are found mainly in sputum and oral samples, respectively. Neutrophil elastase, a serine proteinase that destroys pathogens during infection, is known to be found in high levels in CF patients, while salivary α-amylase is equally present in both the CF and healthy population. By comparing concentrations of neutrophil elastase and salivary α-amylase, we can calculate the level of contamination for each sputum and oral sample. Levels of contamination can be used for future reference in regards to sample procuring procedures, while samples that show less contamination can be sequenced to identify the strains of bacteria present during a cystic fibrosis infection.

Methods

In all wells, specific fluorogenic substrates were prepared as targets for the enzymes being tested. The cleavage of the substrates by the enzymes, emitted fluorescence from the substrate was measured in the NE assays, the Suc-LLVY-AMC peptide was used, while in SA assays, a solution of starch-based conjugate and solvent was used. Microtiter plates were used to hold the samples, each with an area of 1.0 mm2 was then diluted by a factor of 200. 25 μL of each diluted sample was pipetted into 25 μL of specific substrate in triplicate wells to mitigate error. Assays were then analyzed by the assay reader for amount of RFU after 150 seconds of incubation. The data output was then organized, averaged, and analyzed for possible correlations.

Results for NE/SA Assays

It can be seen that sputum samples generally show a strong presence of neutrophil elastase that is significantly larger than the positive control. In addition, mouth rinse that was tested for NE gave weak signals of the enzyme’s presence as expected. Thus, we conclude that levels of NE differ in CF patients due to the varying levels or outright absence of inflammation and infection at the time of testing. In addition, sputum samples had a large range of salivary α-amylase presence. We conclude that this variation may be attributed to the unpredictable nature of sputum procurement; that is, patients produce a variable amount of saliva when dispensing sputum. However, sputum on average had higher levels of SA than mouth rinses, possibly because the mouth produces more saliva when making the effort to cough. Negative controls in both assays showed little enzyme activity as expected. Furthermore, the inclusion of blinds in the experiment provided an additional control group; the resulting data shows a relatively stable amount of SA and decreasing amounts of NE as the saliva concentrations increases. We attribute this to the fact that sputum may contain SA that compensates for changes in saliva concentration.

Analysis and Conclusions

The difficulty of obtaining uncontaminated sputum samples is problematic, and all samples are reduced through changes in the procedure. Nevertheless, we are hopeful that our work can facilitate this to the fact that sputum may contain SA that compensates for changes in saliva concentration. We would first like to thank Dr. Anthony O’Donoghue for providing mentoring and valuable assistance during our stay at the Skaggs School of Pharmacy and Pharmaceutical Sciences. Only with his unfaltering guidance and patience has this project succeeded. We would also like to thank the rest of his lab, specifically Dr. Brian Suzuki, Sandeep Adam, Zhenze Jiang, and Steven Wang for providing a safe and amicable working environment.

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References
