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Cystic Fibrosis, an Alternative Ferripyoverdine Receptor, Probable Remedy?

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Introduction

Cystic Fibrosis, a common autosomal, recessive hereditary disease which affects many different parts of the body including the lungs, gastrointestinal tract, pancreas, reproductive organs and sinuses is caused by mutation in a gene called the Cystic Fibrosis Trans-membrane Conductance Regulator (CFTR), a gene known to help create sweat, digestive juices and mucus (Boyle and Boeck, 2013) . Two copies of this gene are found in the system of healthy individuals and only one gene is sufficient enough to prevent cystic fibrosis. It has been documented that approximately 3.3% of white Americans, or nearly 10 million persons, are carriers of a CFTR mutation; the carrier state is also found in 2.2% of Hispanic persons, 1.5% of African American persons and 1.1% of Asian American persons, with a homozygous prevalence in the United States of approximately 1 per 3500 white persons (Hernanz-Schulman, 2012, Cystic Fibrosis Foundation Report, Plant et al. 2013).

Two prevalent theories surrounding this gene mutation disorder have been explained some decade ago and they are termed the “high salt hypothesis” and the “low volume hypothesis “. CFTR gene encodes a protein anchored to the outer membrane of cells in the sweat glands, lungs, pancreas and other surrounding organs; this protein crosses the membrane and acts as a channel connecting the inner part of the cell (cytoplasm) to the surrounding fluid. This channel is primarily responsible for controlling the movement of negatively charged chloride ions outside the cell into the cell and as such a dysfunctional CFTR would mean an accumulation of these negatively charged ions outside the cells, where also are present positively charged ions like sodium, an electric attraction occurs between sodium and chloride ions and a combination of this results in the formation of salt which is lost in high magnitude in the sweat of individuals suffering from cystic fibrosis disease as compared to healthy individuals, this explains the “high salt hypothesis”. On the other hand, the low volume hypothesis supports identical high salt concentration in healthy individuals and cystic fibrosis patients; this hypothesis also states that lack of the CFTR gene enhances sodium absorption accompanied by chloride from the airway surface liquid which passes either through a second pathway (non-CFTR) or paracellularly. The airway surface cells form a monolayer that is leaky to water and as such increased absorption of salt and water in cystic fibrosis collapses the cilia and hence a ceasation in normal ciliary function which include movement of mucus, and it is this ceasation that forms the basis of lung colonization by bacteria since the normal vehicle that moves bacteria out of the lungs cease to function. However, a common feature of these propositions is that majority of the damages observed in cystic fibrosis is due to blockage of the narrow passages of affected organs with dehydrated and thickened secretions (Smith et al. 1996, Masui et al. 1998, Hassett et al., 2002).

Bacteria therefore find a home in these secretions or mucus which collects in the small airways, this mucus protects bacteria from the direct attack of the host immune system and in addition, within this mucus, bacteria biofilm also develop allowing them to create a kind of niche for themselves.

Majority of the bacteria found to colonize and infect the lungs of cystic fibrosis patients at different stages are Staphylococcus aureus, Hemophilus influenzae (at initial stages) and over time by Pseudomonas aeruginosa and Burkholderia cepacia (O´ Malley, 2009) these bacteria tend to develop special characteristics that enable them adapt and thrive in the environment within the lungs.

Pseudomonas, for example have been observed to develop characteristic features permitting the formation of large mucoid colonies composed of alginate (involving the algD genes), which is the mucoid exopolysaccharide consisting of a repeating polymer of mannuronic and glucuronic acid and it is this alginate slime that forms the matrix of the Pseudomonas biofilm which anchors the bacteria cells to their environment and protects them from the host immune response and administered antibiotics. Other features of Pseudomonas aeruginosa isolates from the lungs of cystic fibrosis patients include the possession of several virulence factors like exoenzyme S which has an ADP-ribosylating activity, and encoded by the

exoS gene, has been suggested to probably impair the function of phagocytic cells in the blood stream and internal organs thereby making preparation for invasion by Pseudomonas aeruginosa possible , the exotoxin A encoded by the toxA gene is proposed to inhibit protein biosynthesis and in addition possesses necrotizing activity at bacteria colonization site and it is therefore thought to contribute to the colonization process , two other extracellular proteases which also contribute to virulence are the lasB elastase and alkaline protease. lasB elastase, a zinc metalloprotease encoded by the lasB has an elastolytic activity on the lung tissue where it lyses fibronectin to expose receptors for bacterial attachment on the mucosa of the lung, elastase is also known to disrupt the respiratory epithelium and interferes with ciliary function, the alkaline protease on its own interfere with fibrin formation and also possesses a lytic activity. In addition to the mentioned virulence factors, P. aeruginosa also has other soluble proteins involved in invasion, these include cytotoxin, a pore forming protein having cytotoxic effect on eukaryotic cells, a phospholipase and a lecithinase both of which possesses haemolytic activity and act synergistically to break down lipids and lecithin. An extracellular neuraminidase also found in P. aeruginosa has been proposed to play an important role in implantation of the bacterium. Several cystic fibrosis isolates tested are in possession of these genes (Lanotte et al., 2004) whose products are responsible for the numerous toxins and virulence factors produced by this notorious gram negative opportunistic pathogen, majority of which are involved in the evasion of the host immune system.

An interesting and important characteristic of this species of gram negative bacteria is their ability to thrive in various ecological niches, which “may” be attributable to their ability to chelate iron which is an essential element for microbial growth and also utilise heterologous iron bearers when in association with other micro-organisms in conditions of iron limitation.

Pseudomonas aeruginosa has a special means of obtaining this essential element. Humans for instance have extracellular and intracellular proteins specialised in trapping iron within its system, examples of which are lactoferrin and transferrin , these proteins help to reduce the availability of free iron thereby limiting microbial access to it, however, in situations of iron limitation such as observed within the human system, pathogenic bacteria like Pseudomonas aeruginosa, a known pathogen of cystic fibrosis patients in which it can cause chronic colonisation of the lungs, secrete low molecular weight compounds called siderophores, these compounds are highly efficient iron strippers, able to compete for and bind any available iron (Neilands,1995) which is subsequently utilised for growth and propagation of virulence within a mammalian host , this kind of behavioural exhibitions are also observed in the external environment.

The two major siderophores secreted by Pseudomonas aeruginosa are Pyoverdine and Pyochelin, pyoverdine, in addition to being a strong chelator of iron is also known to be essential for virulence of P. aeruginosa (Meyer et al., 1996) based on the failure of pyoverdine deficient mutants to demonstrate virulence in burned mice, showing that this compound is of great importance to these species of bacteria.

Large sized complexes are formed between these siderophores and iron and there is need for the internalisation of these complexes so that the bacteria can make use of chelated iron, however, the structure of the cell wall of the gram negative bacteria plays a special role in this internalization process. Diffusion across the gram negative cell membrane is mediated by porins allowing free transport of small size molecules and ions, since the size of these complexes are of several orders of magnitude and concentration of these complexes in the external media is low, bacteria utilizes high affinity receptor proteins located on its outer membrane to initiate the transport process, these receptor proteins are proposed to be used by bacteria to concentrate these complexes at the cell surface and eventually facilitate their uptake into the internal milieu. Several of these receptors have been cloned and identified (Poole et al., 1993) and a number of such is present in the P. aeruginosa genome.

In P. aeruginosa, three siderophore receptors namely fpvA I, II and III exists, each binding its cognate ferri-siderophore ligand (Poole et al., 1993; de Chial et al., 2003).In addition to the three cloned siderophore receptors (fpvA I, II and III), an alternative receptor called fpvB was identified (Ghysels et al., 2004) and identification of this receptor was made possible following the creation of a deletion mutant of an fpvA wildtype Pseudomonas aeruginosa strain and it was observed to be responsible for residual ferri-pyoverdine transport and at the time a 562bp of the product of this gene was amplified. Further work by using experimental PCR procedures (Osayande JO, 2009) has led to the amplification and sequencing of about 2.5kb size fpvB gene in several environmental

and clinical isolates of Pseudomonas aeruginosa and the sequence of this gene in the various isolates tested was highly conserved (even though one of the strain, strain Mi162 see figure 2 showed a little variation, however the same primer sets were used to amplify and sequence the 562bp fpvB gene in this strain) and results show that about 93% of the P. aeruginosa tested are in possession of this gene, this goes to show that this gene maybe yet another important "not-to-do-without" component of the genome of these species of bacteria and if this is true, may open a new avenue for drug discovery for treatment of bacteria (especially Pseudomonas aeruginosa) infections in cystic fibrosis, which on till now, is still striving for complete cure.

Various drug discovery methods in the recent past has involved designing drugs on the basis of iron transport-mediated drug delivery agents (Roosenberg et al., 2000) such as shown below (Figure 1) and detailed biological studies demonstrate a positive indication that this concept of microbial iron transport siderophore- mediated drug delivery, exploiting the active ferric siderophore transport systems is very effective, this concept involves attachment of synthetically made siderophores to drug conjugates (antibiotics) and when microbes recognize these siderophore components as iron delivery agent assimilate the conjugate and in effect are terminated, however, many pathogens recognise and utilize only certain siderophores and such conjugates are thought to have selective antimicrobial activity and apart from this problem of selectivity in antimicrobial activity, third generation antibiotics like the aminoglycosides (penicillins and fluoroquinolones) known to be bactericidal for P. aeruginosa have been extensively studied to require an active transport internalization mechanism in order to exert their bactericidal effect (Hassett et al., 2002; Roosenberg et al., 2000).

Therefore, to further narrow the incidence of selective antimicrobial activity and antibiotic uptake or internalization problems, I am of the opinion that an alternate mode of drug delivery system which encompasses a ¨synthetic receptor¨ (in this case fpvB found in and recognised by about 93% of Pseudomonas aeruginosa strains) linked to a synthetic siderophore- Iron- drug conjugate would go a long way in minimising both incidences. Majority of the drugs known to be effective against isolated biochemical targets become therapeutically ineffective because of their inability to permeate a deleterious cell, this ¨synthetic receptor¨ (fpvB) may speed up the uptake of this drug combination because it "may" quickly be recognised along with siderophore and attached iron.

This opinion is based on the one hand, the fact that the fpvB gene has been shown to confer some ability to take up and utilize existing pyoverdine types (in effect, the types 1 and III pyoverdine) on some clinical and environmental P. aeruginosa isolates (Ghysels et al. 2004), irrespective of the pyoverdine group (three siderovars are known, these include pvdI, pvdII and pvdIII) to which they belong .

On the other hand, since bacteria use considerable metabolic energy to synthesise these receptors and their own siderophores (Buchanan, 2005), they will gladly make use of iron linked to an already made siderophore, which in addition is linked to an already made receptor (¨synthetic receptor¨ fpvB) further linked to an antibiotic and in trying to conserve energy, they utilize these drug conjugates and are ultimately terminated.

Additional information; Source: University of Pittsburgh

Synthetic amino acid sequences called eCAPs are able to push through the outer layers of antibiotic-resistant biofilms, to destroy the entire bacterial community. Researchers say the discovery is especially promising for potential drug treatments for cystic fibrosis. (Credit: UPMC/U. Pittsburgh Schools of the Health Sciences)

"As a result of studying these proteins, we discovered novel structures that turn out to work very well against bacterial infections, including the complicated bacterial populations in lung infections in cystic fibrosis patients."

Cystic fibrosis is a genetic disorder that leads to thick, viscous secretions in the lungs and other organs in about 30,000 children and adults in the United States, according to the Cystic Fibrosis Foundation.

Lung infections resistant to antibiotics often are deadly for people with cystic fibrosis. About 80 percent of cystic fibrosis patients have at least one antibiotic-resistant infection in their lungs by age 18.

"Infections with progressively resistant bacteria in the lung shorten the lives of people with cystic fibrosis," says Joseph M. Pilewski, co-director of the Adult Cystic Fibrosis Center.

“What happens is the genetic defect predisposes patients to infections that drive the production of mucus that then blocks the airways and makes it difficult to breathe.”

As reported in the journal Antimicrobial Agents and Chemotherapy, Montelaro and colleagues found that a particular sequence of amino acids on the tail end of HIV allow the virus to “punch into” and infect cells. The team manufactured a synthetic and more efficient version of this sequence—called engineered cationic antimicrobial peptides, or “eCAPs”—that laboratory tests have shown to rapidly destroy bacteria that are otherwise resistant to most standard antibiotics.

The eCAPs can be assembled in a laboratory setting from the amino acids arginine and tryptophan and manufactured to the shortest effective length, giving the resulting antibiotic treatment maximum potency while reducing costs.

“We have an unmet clinical need for treatment of hospital-acquired infections where the bacteria are extremely resistant to antibiotics,” says co-author Yohei Doi, assistant professor of medicine.

“We have patients with no treatment options left. The fact that these eCAPs are completely engineered puts them at an advantage because they can be manufactured easily, and they give us some hope for a quick-acting treatment in these dire circumstances.”

Traditional antibiotics typically work by poisoning important metabolic processes after being taken up by the target bacteria, a process that may take hours, or days, to clear a bacterial infection. In contrast, the eCAPs are specifically attracted to the surface of target bacteria where they disrupt the bacterial membrane, causing death within seconds, or minutes.

Laboratory tests indicate that the eCAPs work well against biofilms, which are bacterial communities that develop very high levels of resistance to antibiotics by working together to protect the film’s inner bacteria from traditional treatments. The eCAPs seem to push through the outer layers of biofilms to destroy the entire bacterial community.

“It’s like a pin bursting a balloon; it’s a very rapid action,” Montelaro says. “While cystic fibrosis patients are our initial target and a very high-priority target, we also could look at infections associated with burns or indwelling medical devices, such as venous catheters.

“We could even look to the biodefense realm, in terms of a rapid, handheld nebulizer treatment that soldiers could use in the case of exposure to a bioterrorism agent.”

The researchers have taken out several US and international patents on the discovery. The National Institutes of Health funded the study.

Source: University of Pittsburgh

From the on-going discussion above, drug discovery based on iron mediated transport provides an additional dimension in the era of bacterial antibiotic development and following my opinion, a synthetic receptor drug conjugate : Receptor (fpvB)-Siderophore -Iron- linker- drug agent (antibiotic) , will go a long way in reducing symptoms associated with bacterial infection cases in Cystic fibrosis lungs and since the complete sequence of this receptor is now available, a synthetic receptor can be made and subsequently administered to cystic fibrosis patients, many drug activities “may be” restored and or increased by being successfully smuggled into the bacterial cell.

See Illustration:3

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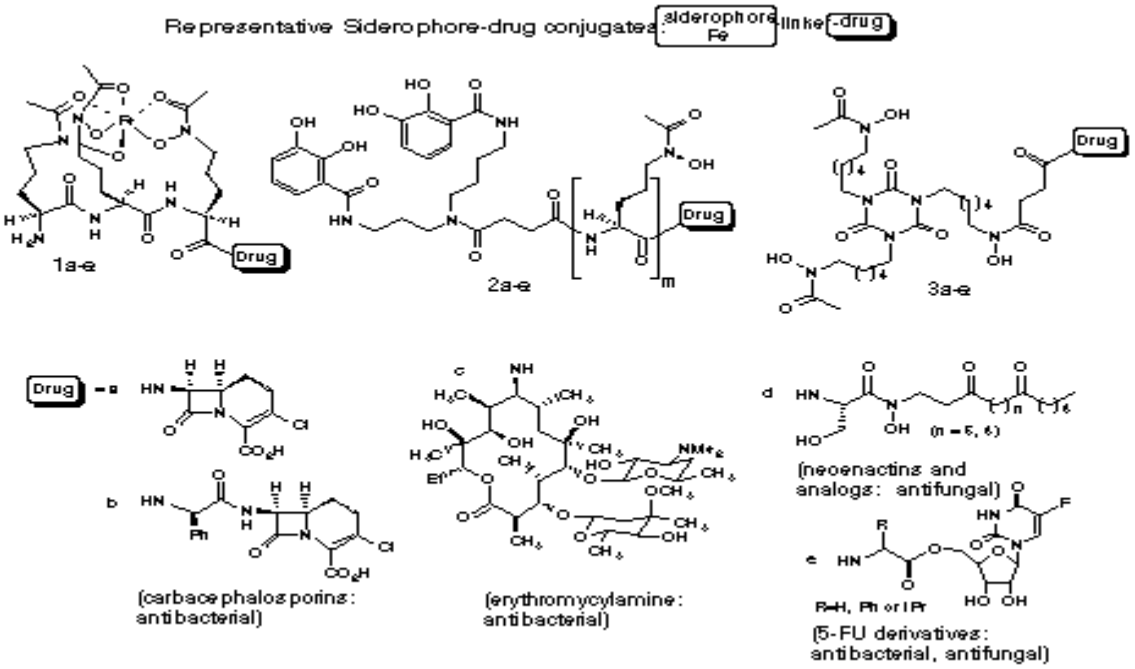
Illustrations

Illustration 1

Figure 1



Conjugate 1a and 1b contain a siderophore component bound to a potent new class of beta-lactam antibiotics called the carbacephalosporins. Conjugate 1c incorporates an erythromycin analog; whereas, conjugate 1d incorporates a siderophore and a novel antifungal agent related to the neoenactins.

Illustration 2

Figure 2

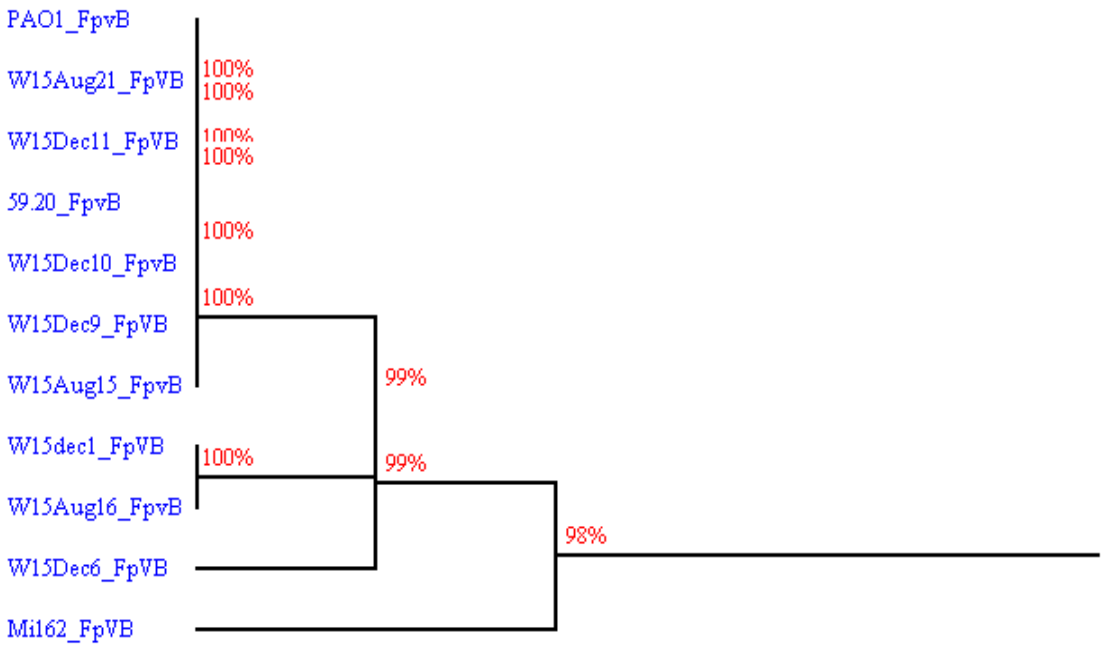
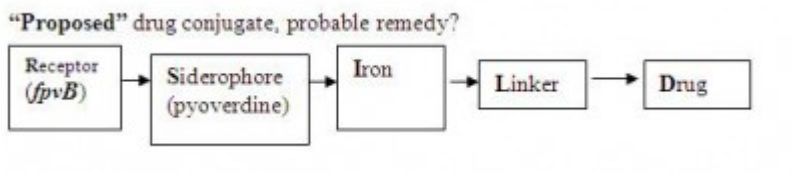


Illustration 3

Figure 3



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