Respiratory Pharmacology: Treatment of Cystic Fibrosis

Dr. Tillie-Louise Hackett
Department of Anesthesiology, Pharmacology and Therapeutics
University of British Columbia

Associate Head, Centre of Heart Lung Innovation, St Paul’s Hospital
Tillie.hackett@hli.ubc.ca
Aims of Lecture

- Define Cystic Fibrosis
- Epidemiology
- CFTR Mutation and classes of mutation
- Defects in lung function in CF
- Treatment
Cystic Fibrosis: Epidemiology

- Term refers to **scarring** (fibrosis) and cyst formation in pancreas - 1930s

- is a **lethal**, chronic, **inherited** disease that harms the respiratory and digestive systems.

- It is the **most common** life-threatening disease **inherited** in the Caucasian population

- 4% of people of European descent are carries for one allele for CF
  - Incidence: 1 in 2000-3000
  - Asia and Africa: 1 in 90,000
Cystic Fibrosis: Clinical Features

Old Proverb 1800 “Woe to the child which when kissed on the forehead tastes salty. He is bewitched and soon will die”

- *affects epithelia in multiple organs*
  - chronic lung infections and inflammatory destruction of lungs
  - nutritional abnormalities due to gastrointestinal obstruction and lack of fluid secretion by intestinal cells
  - pancreatic insufficiency
  - **thick, viscous mucus**
    - Block airways
    - Blocked pancreatic ducts

- Male **infertile** due to congenital malformation of vas deferens

- **Excessive salt loss** from the sweat glands leading to heat exhaustion and dehydration in hot weather and fever
CFTR

- Defect in a gene, called the cystic fibrosis transmembrane conductance regulator (CFTR)

- CFTR is the main **chloride channel** in **epithelia** of various tissues

- ENaC - epithelial sodium channel
- CFTR – chloride Channel
- AQP - aquaporin (water channel)

Water “follows the salt (NaCL) gradient” by **osmosis**
**CF pathophysiology**

- **Fluid / volume secretion problem**
  - In CF, the defect in CFTR causes **decreased Cl- ions**
  - **Inhibits** passive flow of Na$^+$ through tight junctions, actually cell compensatory influx of Na$^+$ to maintain electroneutrality
  - **Inhibits H$_2$O** transcellular flow, water influx and dehydration of mucus

---

Sticky mucus traps **Bacteria**, difficult to Remove by cilia of the lung epithelium
Biofilms

Attachment 1

Growth 2

Detachment 3

Cystic fibrosis is like drowning on the inside.
CFTR is the main chloride channel in epithelia of various tissues.

Epithelial perform diverse functions:

- 1) water or **volume-absorbing** (airways and intestinal tract)
- 2) **Salt-absorbing** (sweat ducts, lung)
- 3) water or **volume-secretting** (pancreas, lung)

All processes involve chloride ion transport; **disruption** of this transport in cystic fibrosis leads to **multiple effects**.
CFTR Mutation

- A big gene (250 kb, 27 exons) located on chromosome 7
- Over **1500 different mutations** in the CFTR gene

![Diagram of CFTR Protein Structure]

Positive charge of Arg = electrostatic barrier

Cl⁻ to Na⁺ permeability ratio = 150 (without Arg352 = 15)

2 **membrane spanning domains** (MSD): anchor protein to the plasma membrane and form the ion channel

2 **nucleotide-binding domains** (NBD-1, NBD-2): hydrolyze ATP and control ion channel gating (opening and closing)

1 **Regulatory domain:** controls activation of CFTR
Classes of CFTR mutations

- **Class I:** Defective protein production (premature stop codon). No or little CFTR protein is produced (5%).

- **Class II:** Defective trafficking (DF508) results in misfolding of protein and degradation in endoplasmic reticulum (75%).

- **Class III:** Mutations in regulatory regions, protein reaches surface but remains closed.

- **Class IV:** Mutations in the membrane-spanning regions, unable to move CL- ions efficiently (Arg 352).

- **Class V:** Reduced synthesis of functional CFTR, unstable mRNA
In Caucasians the ΔF508 mutation is responsible in ~70% of CF cases

This deletion causes loss of the amino acid **phenylalanine**
Located at position 508 in protein; therefore deltaF508
The most common CFTR mutant ΔF508, is a class II defect.

The defective protein retains substantial chloride-channel function in cell-free lipid membranes.

When synthesized by the normal cellular machinery, however, the protein is rapidly recognized as misfolded and is degraded shortly after synthesis, via proteasome degradation pathway at endoplasmic reticulum (ER).
Relationship of clinical features to CFTR function

<table>
<thead>
<tr>
<th>% normal CFTR function</th>
<th>clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1</td>
<td>pancreatic insufficiency and below</td>
</tr>
<tr>
<td>&lt; 4.5</td>
<td>pulmonary infection and below</td>
</tr>
<tr>
<td>&lt; 5</td>
<td>positive sweat test and symptoms below</td>
</tr>
<tr>
<td>&lt; 10</td>
<td>congenital absence of vas deferens</td>
</tr>
<tr>
<td>10-49</td>
<td>none</td>
</tr>
<tr>
<td>50-100</td>
<td>none</td>
</tr>
</tbody>
</table>

- 34% of patients reach adulthood
- 10% live past age of 30
- Average life expectancy – was 20 years
Question

Why is there such a high frequency of CFTR heterozygotes?

- Overstimulation of CFTR in intestinal epithelial cells by bacterial toxins leads to secretory diarrhea

- Toxins (such as cholera) activate the protein kinases that “prime” the CTFR channel

- Cholera is a global health problem resulting in 3 million deaths per year of children under the age of 5
Overview of the organization of lung cells and their functions

* The epithelial lining of tissues in the digestive system share a similar physiology
Lung Defences

- Human airway contains two distinct aqueous layers

- 1) **Mucus layer** – traps inhaled bacteria and foreign particles

- 2) **Airway surface liquid** (ASL) – provides microenvironment for beating cilia to clear mucus layer with assistance of coughing
  - ASL is also rich in proteases, antibiotics & oxidants
  - **Defensins**: small peptide molecules (12-50 aa) containing positively charged and hydrophobic residues.
  - Kill microbes via membrane disruption
  - Their non-specific action makes it difficult to acquire resistance

High salt inactivates defensins
Why is this a problem in the Pancreas?

- One of the main functions of the pancreas is the secretion of digestive enzymes

- Low fluid secretion leads to thick mucus which prevents enzymes from reaching the intestines:
  - Enzymes are retained in the pancreas and eventually destroy all pancreatic tissue
  - Lack of digestive enzymes lead to poor nutrient absorption, weight loss etc
CF lung Disease

- Lungs are plagued by persistent bacterial infections

- **Colonization** occurs early and is nearly impossible to eradicate

- **Biodiversity** of bacteria decreases
  - Main culprits: *P. aeruginosa*, *B. cepacia*, *S. aureus*

- Lung tissue eventually destroyed by onslaught of immune cells (**Neutrophils**) that respond to the infections
Cystic Fibrosis: Current Treatments

Current median age of CF patients is in high 30s

- Medical therapy for clearance of pulmonary secretions
  - Hypertonic saline
  - Physiotherapy
  - Prevents bacterial colonization of mucus

- Pancreatic enzyme replacement

- Adequate nutrients
Cystic Fibrosis: Current Treatments

- **Antibiotics** (tobramycin) – used to control Pseudomonas infections and can be aerosolized for convenient administration

- **Smooth muscle relaxants** (albuterol) – opens airways for easier mucus clearance

- **Anti-inflammatories** – to reduce pulmonary inflammation by immune cells

- **Dnase** – DNA from dead cells and bacteria contribute to thickness of mucus, DNA cleaving enzyme helps to thin mucus
Treatments specific for different classes of CF mutations

- Class I (no protein synthesis)
  - **Gentamicin**: an aminoglycoside antibiotic
  - suppresses premature termination of CFTR mRNA when early stop codons are present – allows some “readthrough”

- Class II (folding and trafficking problems)
  - **deoxyspergualin** competes with Hsp70 chaperone protein which targets for degradation
  - less binding allows more at the surface.
In January 2012, the FDA approved a new drug for patients with G551D (glycine to aspartic acid), Class III mutation (4-5% of CF patients).

The drug, called Kalydeco (VX-770) is a potentiator of CFTR, improves Cl⁻ transport through the channel.
Questions
Panel B: In individuals with CF, the defect in CFTR protein causes decreased secretion of Cl− ions $\Rightarrow$ compensatory influx of Na+ ions to maintain the electroneutrality $\Rightarrow$

Increase in the osmolarity inside the cell $\Rightarrow$
water influx $\Rightarrow$
dehydration of the mucus $\Rightarrow$
sticky mucus traps bacteria, difficult to remove by cilia of the lung epithelium
Classes of CFTR mutations

- **Class I**: Premature termination codons (truncated proteins) and splicing abnormalities lead to severe CFTR reduction (5% of total mutations).
- **Class II**: Deletion of Phe codon (DF508) results in misfolding of protein and degradation in endoplasmic reticulum (75% of mutations).
- **Class III**: Mutations in regulatory regions, protein reaches surface but remains closed (5% of mutations).
- **Class IV**: Mutations in the membrane-spanning regions, unable to move Cl- ions efficiently (15% less severe disease).
Secretion of NaCl and H₂O in normal epithelial cells

- occurs in pancreas, intestine, and submucosal glands of airways

Water “follows the salt” by osmosis