Structure and Function of Salivary Proteins

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“Death is caused by swallowing small amounts of saliva over a long period of time.”
George Carlin
American stand-up Comedian, Actor and Author. b.1937

Outline

• Saliva 101
  – Salivary composition
  – Salivary flows
  – Xerostomia and treatments
  – Basic functions of saliva and salivary proteins
• Advanced Salivary Technology
  – Medical diagnostic value of saliva
  – Proteomic analysis of saliva and applications

Basic salivary components

• 99% H2O
• Ions: Na⁺, K⁺, Ca²⁺, Cl⁻, H₂CO₃⁻, PO₄³⁻
• Proteins: Enzymes, Immunoglobulins, glycoproteins, peptides.
• Others: glucose, urea, NH₃

Whole Saliva

• Fluids (and mucous) from
  – Salivary glands
  – Gingival fold
  – Oral mucosa transudate
  – Nasal cavity/pharynx
• Bacterial components
• Food
• Epithelial/blood cells
• Traces of medications/chemical products

Structure of a Salivary Gland
Salivary Production

- 80-90% is stimulated saliva
- 1-1.5 L per day
- Salivary Flow (SF): ml/min
  - Normal: 1-3 0.25-0.35
  - Low: 0.7-1 0.1-0.25
  - Very Low: < 0.7 < 0.1 (hyposalivation-xerostomia)

Causes of dry mouth

- Iatrogenic
  - Drugs
  - Irradiation
  - Graft vs host disease
- Disease
  - Salivary gland disease
  - Sjogren’s syndrome
  - Sarcoidosis
  - Cystic fibrosis
  - Primary biliary cirrhosis
- Infections
  - HIV
  - Human T lymphotropic virus 1 (HTLV-1)
- Dehydration
- Psychogenic

Drugs associated with dry mouth

- Anticholinergic drugs
- Tricyclic antidepressants
- Muscarinic receptor antagonists for treatment of overactive bladder
- Alpha receptor antagonists for treatment of urinary retention
- Antipsychotics such as phenothiazines
- Diuretics
- Antihistamines
- Sympathomimetic drugs
- Antihypertensive agents
- Antidepressants (serotonin agonists, noradrenaline and/or serotonin re-uptake blockers)
- Appetite suppressants
- Decongestants and 'cold cures'
- Bronchodilators
- Skeletal muscle relaxants
- Antimigraine agents
- Benzodiazepines, hypnotics, opioids and drugs of abuse
- H2 antagonists and proton pump inhibitors
- Cytotoxic drugs
- Retinoids
- Anti-HIV drugs such as didanosine (DDI) and protease inhibitors
- Cytokines

What type of treatments do you prescribe for patients with Xerostomia?

Treatment for Xerostomia

- Goals:
  - Alleviate symptoms and prevent complications such as dental caries, gum disease, halitosis, salivary gland calculi, and dysphagia
  - Two major components
    - stimulation of existing salivary flow
    - replacement of salivary secretions.

Electronic Control of Salivation

Apply low energy levels of electrical stimulation to the oral mucosa (oral tissue surface) close to the nerves controlling salivary function. This leads to a higher level of saliva secretion.

The custom made remote control turns "ON" and "OFF" the electrical stimulation.
Stimulation of existing salivary flow

- Physio-chemical stimuli
  - Sucking on something
- Sugar-free candies
  - Salivasure®: Scandinavian candies containing malic acid
- Sugar-free gum
  - Containing various sweeteners such as aspartame, saccharin, and sorbitol

- Drugs
  - Pilocarpine (Salagen®)
  - Cevimeline (Evoxac®)

Pilocarpine

- Salagen®
  - A muscarinic agonist that stimulates predominantly muscarinic M3 receptors
- Doses
  - 5 mg three or four times daily
- Side effects
  - sweating, abdominal pain, flushing, increased urination

Cevimeline

- Evoxac®
  - A derivative of acetylcholine with a higher affinity for muscarinic M1 and M3 receptors on the lacrimal and salivary epithelium (than for receptors on heart tissue).
- Doses
  - 30-60 mg three times daily
  - alleviate the symptoms of dry mouth, dry eyes, and stimulate salivary flow
  - 30 mg usually is just as effective as 60 mg
- Side-effects
  - excessive sweating, nausea, rhinitis, diarrhea, and visual disturbances
  - contraindicated in patients with asthma, narrow-angle glaucoma, or iritis

Why do we need saliva?

"The first kiss I had was the most disgusting thing in my life. The girl injected about a pound of saliva, into my mouth, and when I walked away I had to spit it all out."
Leonardo DiCaprio
(American Actor, b.1974)

Basic Functions of Saliva

- Taste
- Protection and Lubrication
- Dilution & Cleaning
- Buffering
- Protection of Enamel
- Pain Control?
- Digestion
- Tissue Repair
- Antimicrobial Properties
- Film & Calculus Formation
Submandibular Gland

Is saliva hypotonic, isotonic, or hypertonic?

Taste

- Saliva is hypotonic.
  - Low levels of glucose, Na⁺, Cl⁻, and urea
  - Allow easy dissolution of substances for taste buds
- Gustin
  - A salivary protein
  - Necessary for growth & maturation of taste buds

Why Saliva is Hypotonic?

Model 1

Baseline Membrane
- Na⁺/K⁺ ATPase
- Na⁺-K⁺-2Cl⁻ (NaKCCl) Cotransporter
- Ca²⁺ activated K⁺ Channel
  - Open with stimuli (increase Ca²⁺ in the cell)
Apical Membrane
- Ca²⁺ activated Cl⁻ Channel
  - Open with stimuli (increase Ca²⁺ in the cell)

Model 2 & 3

Model 2
- Cl⁻/HCO₃⁻ exchanger
- Na⁺/H⁺ exchanger
Replace NKCCl
- Carbonic Anhydrase
  - CO₂ into H⁺ and HCO₃⁻
Model 3
- HCO₃⁻ instead of Cl⁻ is excreted into the acinar lumen

Protection & Lubrication

- Seromucosal covering
  - Mucins (hi Carb proteins)
- Lubricating
  - Masticatory/Speech/Deglutition
- Dehydration protection
- Maintaining salivary viso-elasticity
- Controlling adhesion/colonization of microorganisms
Dilution & Cleaning
- Low in sugar
  - ~ 0.5-1 mg/100ml
  - Allow limited biofilm formation or bacterial growth
- Mechanical cleansing
  - depending on SF
    - Higher SF, better cleansing

Buffering
- Prevent (selectively) bacterial colonization
- Neutralize acids from bacteria
  - Prevent enamel demineralization
- Buffering capacity
  - Thickness of biofilm
  - Amount of bacteria
- Proteins-Sialin
- Urea
- NH₂--Renal insufficiency children- No caries
- HCO³ Stimulated
- PO₄³ Unstimulated

Integrity of Enamel
- Modulating re-/de-mineralization
- Depending Ca²⁺, PO₄³⁻, F⁻ and pH of saliva
- Ca²⁺ varies with the SF, not affected by diet
  - Bind to other inorganic ions (ionized form)
  - Bind to organic ions/proteins (non-ionized form)
- H₃PO₄, H₂PO₄⁻, HPO₄²⁻ and PO₄³⁻: depend on salivary pH (more in unstimulated) and SF
- F⁻: depends on consumption
- pH:
  - Normal salivary pH is from 6 to 7
  - Varies with the SF, from 5.3 (low flow) to 7.8 (peak flow)

Pain Control?
- Opiorphin is a chemical compound isolated from human saliva.
- Initial research with mice shows the compound has a painkilling effect of up to six times that of morphine.
- It works by stopping the normal breakdown of natural pain-killing opioids in the spine, called enkephalins.
- It is a relatively simple molecule that should be possible to replicate and synthesize in large quantities

Protein Secretion
- A model for the mechanism of cAMP- and Ca²⁺-dependent amylase secretion in rat parotid acinar cells: cAMP stimulates the formation of docked/primed secretory granules and enhances the effect of Ca²⁺ as the trigger for fusion/secretion

Digestion
- α-amylase (ptyalin)
  - 40-50% of all salivary proteins produced by salivary glands
  - 80% of amylase in saliva is produced by parotid glands
  - Indication for proper salivary function
Tissue Repair

- Coagulating factors
- Epidermal growth factor (EGF)

Anti-microbial proteins

- Muc5B (MG1)
- MUC7 (MG2)
- Immunoglobulins
- Proline-rich proteins
- Cystatins
- Histatins
- EP-GP
- Agglutinin
- Lysozyme
- Lactoferrin
- Lactoperoxidase
- Cathelicidin
- Defensins

Immunoglobulins

- Secretory IgA
  - 5 – 15% of total salivary proteins
  - Produced by B lymphocytes
  - sIgA is not opsonizing, as there are not cytotoxic T cells in saliva
  - Neutralize viruses, bacterial, and enzyme toxins
  - Serves as an antibody for bacterial antigens and is able to aggregate bacteria, inhibiting their adherence to oral tissues

Lysozyme

- Hydrolyze the cellular wall of some bacteria
- Strongly cationic-activate the bacterial "autolisines"
  - Hydrolyzes bacterial cell wall, making bacteria vulnerable to lysis in hypotonic salivary fluid
- Gram-negative bacteria are more resistant due to their external lipopolysaccharide layer
- May help in bacterial aggregation and inhibition of bacterial adherence

Mucins

- MUC5 10 – 30 MDa
- MUC7 130 kDa

- Comprise about 20% of total salivary protein

- Mucins, e.g. MUC5
  - is very viscous in solution
  - may contribute to physical properties of saliva

Mucins

- Both MUC5 and MUC7 bind to wide variety of bacteria including S. mutans
- Implicated in protection from viruses
- Diversity of function related to diversity of the oligosaccharide side chains
Lactoferrin

- Binds free iron in the saliva causing bactericidal or bacteriostatic effects on various microorganisms requiring iron for their survival
  - *Streptococcus mutans* group
- Provides fungicidal, antiviral, anti-inflammatory, and immunomodulatory functions

Peroxidase (Sialoperoxidase)

- Serves as a catalyst for the oxidation of the salivary thiocyanate ion by hydrogen peroxide into hypothyocyanate - a potent antibacterial substance

Proline-rich proteins

- 15-20% of parotid saliva
- A minor component of unstimulated saliva, but when stimulated 10% of whole saliva
- 36 kDa cationic protein
- Interacts specifically with *F. nucleatum* and may interfere with plaque formation

Proline-rich proteins and Statherins

- Inhibit the spontaneous precipitation of calcium phosphate salts and the growth of hydroxyapatite crystals on the tooth surface, preventing the formation of salivary and dental calculus

Histatins

- A family of related cationic proteins rich in histidine
- Broad antimicrobial activity against bacteria and yeasts
- Synthetic peptides (e.g. P113) has been tested as an antimicrobial component of mouth rinses (Paquette et al 2002)

Parotid Secretory Protein (PSP)

- Parotid secretory protein (PSP) and palate-lung-nasal epithelium clone (PLUNC)
- Novel secretory proteins that are expressed in the oral cavity and upper airways
- Related to bactericidal/permeability increasing protein (BPI)
- Cationic peptides derived from BPI exhibit anti-inflammatory activity
- Inhibited the lipopolysaccharide (LPS)-stimulated secretion of TNF from macrophage cells
- Directly inhibited the binding of LPS to LPS-binding protein
- Can serve as templates for the design of novel anti-inflammatory peptides
How can we use saliva in clinical practice/clinical research?

Use of saliva in clinical diagnosis and therapy

- **DNA microarray technology**
  - The presence of RNA (through cDNA)

- **Mass spectrometry-based proteomic analysis**
  - The presence of Protein

**DNA Microarray**

- A high-throughput technology
- Consists of an arrayed series of thousands of microscopic spots of DNA oligonucleotides
  - Picomoles of a specific DNA sequence or short section of a gene
  - Used as probes to hybridize a cDNA or cRNA sample (target) under high-stringency conditions.
- Probe-target hybridization is usually detected and quantified by fluorescence-based detection
- Measure DNA or use DNA as part of its detection system.
- Measure changes in expression levels or to detect single nucleotide polymorphisms (SNPs)

Wong et al at UCLA

- Gene expression profile in saliva
- Microarray technology
- Over 3000 gene targets
### Disadvantages of DNA microarray

- **Only known genes**
  - Sequence must be known!
  - Novel genes may not be included in the array
- **Depends on availability of mRNA**
  - mRNA may not be stable
  - Limited amount of mRNA
- **Difficulty in interpretation**
  - Bioinformatics

### Intro to clinical proteomics

- Traditionally, we use individual (serum) biomarkers to identify diseases, e.g., cancer and other conditions
  - PSA (Prostate-specific antigen)
  - CA125 (ovarian cancer)
  - Estrogen receptor (estrogen-dependent mammary cancer)
- ELISA (enzyme-linked immunosorbent assays) is often used.
  - Required specific antibodies against particular biomarkers
  - However, single or even multiple biomarkers often do not produce unequivocal answer
    - Sensitivity: they may not be present in a detectable amount in all patients esp. early-stage
    - Specificity: they may also express in non-cancerous tissues

### Proteomic Analysis of Saliva

- **What is proteomic analysis?**
  - large-scale study of proteins, particularly their structures and functions.
  - The word "proteome" is a blend of "protein" and "genome". T
  - The proteome is the entire complement of proteins, including the modifications made to a particular set of proteins, produced by an organism or system. This will vary with time and distinct requirements, or stresses, that a cell or organism undergoes.

wikipedia.org

### Conventional Mass Spectrometry

- Separate proteins by 2D gel electrophoresis
- Subject individual protein band to MS
- Time consuming
- Labor intensive
- Often not reproducible

### 2D gel electrophoresis

- MALDI-TOF
  - Matrix-assisted laser desorption/ionization (MALDI)
  - A soft ionization technique used in mass spectrometry
  - Allow the analysis of biomolecules (biopolymers such as proteins, peptides and sugars) and large organic molecules (such as polymers, dendrimers and other macromolecules)
  - Tend to be fragile and fragment when ionized by more conventional ionization methods
  - Similar to electrospray ionization both in relative softness and the ions produced
  - The ionization is triggered by a laser beam (normally a nitrogen laser). A matrix is used to protect the biomolecule from being destroyed by direct laser beam and to facilitate vaporization and ionization.
MALDI-TOF

- Directly analyze proteins in a sample by mass (molecular weight)
- Profiling
  - Can detect multiple biomarkers simultaneously
  - ELISA can only do one protein

Basic Concept of Cancer Proteomic

- Normal and diseased (cancer) tissues express different proteins
- There are specific proteins (biomarkers) expressed in diseased or tumor tissues that are different from or absent in normal tissues

Principle of MALDI-TOF/TOF MS

SELDI-TOF

- Surface-enhanced laser desorption/ionization
- A variation of matrix-assisted laser desorption/ionization (MALDI)
- Uses a target modified to achieve biochemical affinity with the analyte compound

In MALDI
- Protein or peptide sample is mixed with the matrix molecule in solution and small amounts of the mixture are deposited on a surface and allowed to dry
- Sample and matrix co-crystallize as the solvent evaporates

In SELDI
- Protein mixture is spotted on a surface modified with a chemical functionality. Some proteins in the sample bind to the surface, while the others are removed by washing.
- After washing the spotted sample, the matrix is applied to the surface and allowed to crystallize with the sample peptides. Binding to the SELDI surface acts as a separation step and the subset of proteins that bind to the surface are easier to analyze.
Protein Chips (SELDI-TOF)

Identification & Uses of Biomarkers

Mass Spectrometry-based clinical proteomics

- "Gold Standard" for identification/analysis of individual proteins in proteomic studies
- Can also use to analyze single nucleotide polymorphism (SNP) or post-translational modifications
- Clinical uses in diagnostic and predictive medicine
  - Identification of new Biomarkers
  - Proteomic profiling
    - Multiple protein monitoring with known Biomarkers
    - Allow multivariate analysis of Biomarkers
  - Detection of diseases at their early-stage

Disadvantages

- Difficult to analyze data
- Variations of Control and Case may give false positive in identifying new markers.
- Protein stability & expression
  - Sample collection, storage, processing and handling
  - Day-to-day variation

Thank you