First Time in Human Safety Study of *Streptococcus mutans* Lactic Acid-deficient Effector Strain (A2JM) Administered in Conjunction with Twice Daily Dose of D-alanine Mouthwash in Healthy Adult Male Subjects for Replacement Therapy as an Aid in the Protection Against Dental Caries

Protocol #0311-614
Program Team

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Significance

- Dental caries is the most common chronic disease (5 billion people worldwide)
- Approximately $40 billion was spent in the U.S. in 2003 on dental caries (5% of total health care costs)
- An increasing body of evidence has associated oral infections with systemic diseases, such as cardiovascular disease
Background

- An infectious disease, principally caused by the indigenous plaque bacterium *S. mutans*
- Dietary sugar is metabolized by *S. mutans* to produce lactic acid, which dissolves the mineral portion of tooth enamel and dentine
- Current methods for prevention aim at reducing the levels of *S. mutans* or making the teeth more resistant to acid attack
  - All require patient compliance
Hypothesis

- The presence of a particular microorganism can prevent colonization by a pathogen or keep its numbers below the level required for it to manifest disease.

- This hypothesis forms the basis for an approach to prevent infections called *replacement therapy*.
Replacement Therapy

- Depends on finding or creating an *effector strain* that has 3 essential properties:
  1. It does not cause disease itself
  2. It can persistently colonize the tissue at risk and outcompete disease-causing strains
  3. It is genetically stable
The A2JM Effector Strain: Pathogenic Potential

- The gene for lactate dehydrogenase (\textit{ldh}) was deleted
- Deletion of \textit{ldh} killed the bacteria; compensate for this by adding a supplemental gene for alcohol dehydrogenase (\textit{adhB})
The A2JM Effector Strain: Colonization Potential

- Perform strain construction starting with a human S. *mutans* isolate that naturally produces a bacteriocin capable of killing all other S. *mutans* strains.
The A2JM Effector Strain: Genetic Stability

- LDH deficiency due to a deletion mutation
- Genetic transformation occurs at very low frequencies due to:
  - A naturally occurring insertion mutation in the competence gene, *comC*, which reduces transformation frequency
  - An engineered deletion mutation in another competence gene
- No prophage or known transducing phage
Horizontal Transmission

- Transmission is typically vertical (mother to child)
- The minimal infectious dose for A2JM is 1000-fold higher than for its \( ldh^+ \) parent
- Adult male volunteers colonized with mutacin 1140-producing \( S. \) \textit{mutans} did not transmit this strain to spouses or children over a 15-year period
Additional Safeguard

- A2JM is dependent on environmental D-alanine due to a deletion mutation in alanine racemase (\textit{dal})
Laboratory and Animal Testing of A2JM

- It did not cause significant tooth decay in rats
- It persistently colonized the teeth of rats
- It displaced other *S. mutans* strains
- It was genetically stable in the laboratory and in animals
- It showed no toxicity in acute and chronic (1 year) tests
- It did not disrupt the normal oral flora
Study Design Scheme

A2JM TXT: 5x10^{10} cells applied using swab for 5 min

Screen: N=16 young healthy ♂s and spouses

A: D-alanine mouthwash plus chlorhexidine N=8

Follow-up

B: Chlorhexidine alone N=8

Follow-up

2 wk | 1 wk | 3 mo | 3 mo
Objectives

❖ Primary Objective
  – To assess safety and tolerability of A2JM and D-alanine

❖ Secondary Objectives
  – To estimate stability of A2JM genetic profile
  – To estimate A2JM transfer to partners
  – To determine if A2JM can be eradicated or reduced in numbers below its minimal pathogenic dose
Inclusion Criteria

- Subjects must be male between 21 and 35 years old
- Subject must be in a stable, monogamous relationship
- Both partners must be healthy
- No less than 20 natural, minimally restored teeth
- Partner must remain non-pregnant throughout the trial
- Both subject and partner must have an indigenous mutans streptococcus strain
Exclusion Criteria

- Abnormal baseline physiological findings
- Rheumatic fever, valvular heart disease, SBE
- Oral abnormalities that compromise gingival integrity
- Significant chronic clinical illness (e.g., hepatitis, HIV)
- Children living in the same household
- Employed as food handlers, day care or healthcare provider
Outcome Measures

- Measure frequency and nature of adverse events
- Collect saliva samples from subjects and spouses
  - Determine genetic stability
  - Determine occurrence of horizontal transmission
Advantages of Replacement Therapy

- Lifelong protection from a single treatment
- No patient compliance required
Plaque is a Complex Biofilm