Toxicity and Inflammatory Response to Common Magic Mouthwashes in a 3D Oral Reconstructed Tissue Model

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Introduction

Magic Mouthwash is a general term describing oral rinses prescribed by a doctor for the treatment of pain, inflammation, or infection, commonly as a result of chemotherapy and radiation induced oral sores (oral mucositis). There is no set combination of ingredients for the preparation of a magic mouthwash, rather they are formulated for the individual needs of each patient, selecting from an array of various active ingredients. Although the ingredients most commonly used to formulate Magic Mouthwashes are considered safe as prescribed, the final formulations lack toxicity and inflammatory response data. Four common Magic Mouthwash formulations (MM 1,2,3,4), which vary slightly in active and inactive ingredients, were tested for cytotoxicity and inflammatory response using a novel testing strategy based on a commercially available 3D EpitOral™ tissue model (MatTek Corporation, Ashland, MA), reconstructed from normal human-derived oral epithelial cells. This in vitro model can be used to accurately and reproducibly assess the safety of products designed to treat oral mucosal conditions and thus reduce the burden of clinical studies.

Materials & Methods

Magic Mouthwashes Contain Aqueous Solutions
- Sweeteners (MM 1,2,3,4)
- Antiseptic (MM 1,3,4)
- Anti-Inflammatory (MM 2,3,4)
- Anesthetic (MM 1)

Duplicate MatTek EpitOral™ 3D Tissues
- 4 Magic Mouthwashes (MM 1,2,3,4)
- Negative Control (NC), Water (30 min., 24 hr.)
- Positive Control (PC), 1% TritonX-100 (20 min., 1 hr., 2 hr.)

Clinical Exposure Reflected by Topical Application
- 5 min. MM exposures represent time spent washing
- 30 min. MM exposures mimic clinical exposure before rinsing
- 16 hr. MM exposures mimic a full day of exposure

ELISA Immune Response Assay
- Media collected to quantify cytokines secreted
- IL-1β measured compared to standard
(16 hr. MM and 30 min. NC)

MTT Cytotoxicity Assay
- Extracted formazan salt (purple) indicates cell survival
- Cytotoxicity generally correlates with toxicity
- Provides % Cell Survival and potentially ETs values
- Provides two-way (cytotoxicity) or one-way (immune response) ANOVA used
* p<0.05

Materials & Methods

Results

Magic Mouthwash Cytotoxicity

Magic Mouthwash 1 (MM 1):
- AA: Mylanta (33%)
- AF: Nystatin (0.1%)
- AN: Lidocaine (0.5%)
- AH: Diphendrydramine (0.01%)

Magic Mouthwash 2 (MM 2):
- AA: Hydrocortisone (0.1%)
- AF: Nystatin (0.08%)
- AN: Lidocaine (0.07%)
- AH: Diphendrydramine (0.025%)

Magic Mouthwash 3 (MM 3):
- AN: Lidocaine (0.7%) 
- AH: Diphendrydramine (0.08%)
- AI: Dexamethasone (0.004%)

Magic Mouthwash 4 (MM 4):
- AN: Lidocaine (0.5%)
- AF: Nystatin (0.4%)
- AI: Prednisolone (0.075%)
- AH: Diphendrydramine (0.0625%)

Histology of 3D & Native Oral Tissue

EpiOral™ 3D Buccal Phenotype
- s. disdentum
- s. filamentosum
- s. disdentum
- s. filamentosum

Histology is from MatTek Corporation of an EpiOral™ tissue model.

Conclusions & Future Directions

Magic Mouthwash 1 (MM 1):
- 33% Mylanta, AA used to coat the mouth and increase bioavailability.
- The only basic formulation tested (pH=8).
- The most cytotoxic after 16 hours.
- Significant cell viability loss between short and long-term exposures (30% and 0.06%), and the highest inflammatory response (IL-1β = 44.9 pg/mL).
- The unique pH and concentration of Mylanta™ indicates that the pH and cytotoxicity are likely driven by the antacid.

Magic Mouthwash 2 (MM 2):
- No long-term toxicity was induced by MM 2.
- Magic Mouthwashes 2, 3, and 4 (MM 2, 3, 4) had the anti-inflammatory steroids found in the composition of MM 2, and 4 correlated with reduced inflammatory response when compared to MM 1, which did not contain a steroid.

Future Directions:
- The role of synergism requires further investigation, particularly surrounding the effects of lidocaine on the cytotoxicity induced to the tissues exposed to MM 1.
- The effects of the high concentration of antacid in MM 1 requires additional studies.
- The long exposure time needed to exhibit significant oral toxicity and inflammatory response supports the conclusion that Magic Mouthwashes tested are safe when used as prescribed.

Furthermore, the data promotes the use of non-animal methods based on reconstructed human tissue models to assess the safety of oral products for human use.
- Correlation with clinical endpoints is needed to gain confidence in the in vitro testing platform presented.

Native Oral Tissue

With histology not performed on the MM-treated tissues, these representative images of native and 3D buccal tissue provide visual representation of the similarities between these in vivo and in vitro models.