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Development of a Novel Aspirin Suppository Formulation and Evaluation of the Acetylation of COX-1 Via a HT-29/Caco-2 Cell Absorption Assay Used to Detect the Absorption of Aspirin Formulated With Various Bases and Excipients

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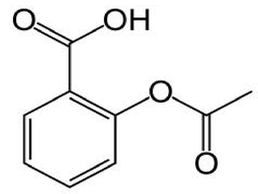


STATEMENT OF THE PROBLEM

Background

Aging Baby-boomer Population

- Aspirin use increases with age.[13] The aging baby-boomer population is resulting in more patients who are not able to take medication orally (NPO).
- Rectal suppositories are the commonly used alternative for NPO patients.

Current Aspirin Suppositories

- Only one is available from Paddock labs. Previous research does not specify if this was the formulation used.
- Very little research is available concerning aspirin suppositories.
- Current research is dated and more foundational in nature rather than comprehensive and adequately powered
  - A study concluded that rectal absorption of aspirin was slow and long in duration whereas a study by Broom stated that serum levels were short-lived.[1]
  - The most foundational were a series of studies from the seventies that involved very small sample sizes and were inadequately powered.[8,14,3]

Mechanism of Action[6]

•Aspirin is hydrolyzed to the active component salicylate by esterases in the GI mucosa, red blood cells, synovial fluid, and blood

•Aspirin irreversibly inhibits cyclooxygenase-1 and 2 (COX-1 and 2) enzymes via acetylation.

•This causes a decrease in the formation of prostaglandin precursors and irreversibly inhibits the formation of thromboxane A_2, thus inhibiting platelet aggregation.

•This action also has antipyretic, analgesic, and anti-inflammatory benefit.

Significance of the Problem

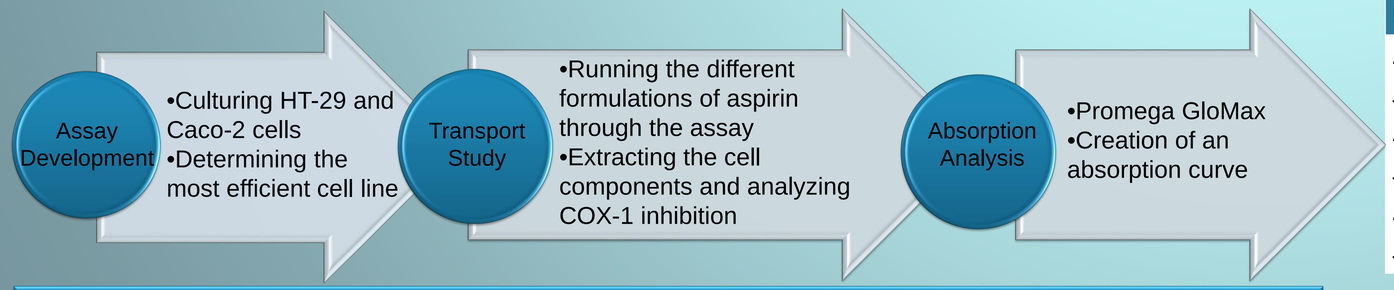
- The demand for dosage forms that accommodate patients who can take nothing by mouth
- The absorption of the current aspirin suppository available is poor and erratic
- There is a need for a better aspirin suppository product to enhance absorption and therapeutic effect.

OBJECTIVES

To create a novel aspirin suppository with increased absorption of cells.

ALTERNATIVE HYPOTHESIS

- The inclusion of zinc and carnosine excipients and selected bases in the formulation of aspirin (or sodium salicylate) suppositories increases the absorption in HT-29 colonic adenocarcinoma cells or Caco-2 cell lines.



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PROPOSED METHODS

Study Design:

- Prospective, pre-clinical in-vitro study to detect aspirin (in suppository formulation) absorption through rectal cells

Sample:

- Cultured HT-29 colonic adenocarcinoma cell line
- Cultured CaCo2 colorectal adenocarcinoma cell line
- Selected cell-line will be cultured for use in the developed assay to test the absorption of aspirin formulated with different bases and excipients

Data Collection:

Phase One: Comparison of commercial aspirin suppository absorption through HT-29 versus CaCo-2 cell cultures.

- Cultured cells in the assay will be seeded to monolayer filter inserts to approximate rectal cells and be the barrier which the aspirin must cross.
- Aspirin that crosses the barrier of the cells will be determined by an absorption reading by the Promega GloMax Multi-Plate reader. [15] This reading will be a measurement of the amount of COX-1 acetylation.

Phase Two: Comparison of aspirin bases and excipients using the preferred cell line.

- Cultured cells (from the preferred cell line) will be cultured and used in the developed assay.[2]
- Aspirin in addition to the chosen bases/excipients will be run through the assay.
- Serum containing the cellular components of the cell line will be placed in well plates and absorption (measurement of COX-1 acetylation in the cells) will be determined by the Promega GloMax. This will be our primary way of measuring the amount of aspirin absorbed.

Measurement:

- The Promega GloMax Multi-Plate reader will be used to generate a standard curve of aspirin absorption using intracellular aspirin concentration (measured by the amount of acetylation of COX-1).
- These absorption curves will be created for each "formula" of aspirin and will be compared to each other and to the curve from the commercially available aspirin suppository formulation.

PROPOSED ANALAYSES

All data will be expressed as mean±standard deviation and will be analyzed using repeated measures ANOVA with Dunnett's post-test, as appropriate (SPSS version). A value of P, 0.05 (two-tailed) is considered statistically significant.

PROJECT TIMELINE

Dates	Expectation
August-December 2013	Research proposal development
January-May 2014	Obtain the assay, reagents, and aspirin
August-December 2014	Assay validation
January-May 2015	Test and evaluate aspirin suppository formulations
August-December 2015	Propose human studies
January-May 2016	Conduct study

LIMITATIONS

- The assay may not fully approximate the actual cells in the rectum.
- We do not know the demand for aspirin suppositories.
- Sensitivity measurements in the assay of acetyl salicylic absorption.
- Unable to use radioactive tagging or isotopes.

FUTURE DIRECTIONS

- A follow up clinical trial will be conducted to compare the efficacy of the novel suppository formulation against the leading formulation for both pain management and cardiac regimens.
- Efficacy of oral versus rectal routes for aspirin can be evaluated with the best absorbed formulations.
- Need assessment research will be conducted to identify which health care settings would benefit most from an improved aspirin suppository formulation.