EFFECT OF EXOGENOUS EPIDERMAL GROWTH FACTOR ON THE NORMAL AND ULCERATED COLON IN RATS

A thesis submitted to the University of Adelaide,
South Australia,
for the degree of Doctor of Philosophy

by

KAREN ANN RIBBONS. BSc.(Hons.) - Flinders University

Department of Obstetrics and Gynaecology,
University of Adelaide,
South Australia. 5000

May 1993
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>THESIS ABSTRACT</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>CHAPTER ONE  LITERATURE REVIEW</strong></td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>3</td>
</tr>
<tr>
<td>The Pathophysiology of Inflammatory Bowel Disease</td>
<td>3</td>
</tr>
<tr>
<td>Introduction</td>
<td>3</td>
</tr>
<tr>
<td>Etiology of Inflammatory Bowel Disease</td>
<td>4</td>
</tr>
<tr>
<td>Normal colon morphology</td>
<td>4</td>
</tr>
<tr>
<td>Disease symptoms</td>
<td>5</td>
</tr>
<tr>
<td>- Ulcerative colitis</td>
<td>5</td>
</tr>
<tr>
<td>- Crohn’s Disease</td>
<td>6</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>7</td>
</tr>
<tr>
<td>Biochemical and functional changes in the colonic mucosa associated with inflammatory bowel disease</td>
<td>8</td>
</tr>
<tr>
<td>- Colonic proliferation</td>
<td>8</td>
</tr>
<tr>
<td>cell proliferation in the normal colon</td>
<td>8</td>
</tr>
<tr>
<td>colonic proliferation in colitis</td>
<td>9</td>
</tr>
<tr>
<td>- Mucosal permeability</td>
<td>10</td>
</tr>
<tr>
<td>- Electrolyte balance</td>
<td>11</td>
</tr>
<tr>
<td>short chain fatty acids</td>
<td>12</td>
</tr>
<tr>
<td>- Colonic mucin</td>
<td>12</td>
</tr>
<tr>
<td>Mediators of colonic inflammation</td>
<td>13</td>
</tr>
<tr>
<td>- T cell activation</td>
<td>13</td>
</tr>
<tr>
<td>- Neutrophil activation</td>
<td>14</td>
</tr>
<tr>
<td>- Macrophage activation</td>
<td>15</td>
</tr>
<tr>
<td>- Eicosanoids</td>
<td>15</td>
</tr>
<tr>
<td>- Summary</td>
<td>17</td>
</tr>
<tr>
<td>Current treatments used in inflammatory bowel disease</td>
<td>17</td>
</tr>
<tr>
<td>- Drug therapy</td>
<td>18</td>
</tr>
<tr>
<td>aminosalicylates</td>
<td>18</td>
</tr>
<tr>
<td>corticosteroid therapy</td>
<td>20</td>
</tr>
<tr>
<td>- Surgery</td>
<td>22</td>
</tr>
<tr>
<td>- Nutrition and Nitrogen balance</td>
<td>23</td>
</tr>
<tr>
<td>- New drug therapies</td>
<td>24</td>
</tr>
<tr>
<td>f-ASA</td>
<td>24</td>
</tr>
</tbody>
</table>
Histological measurements
- Quantitative morphology
  Immunohistochemical localization of PCNA
  Measurement of PCNA labelling index and PCNA labelled crypt fraction
Stability of EGF in the colonic lumen
Measurement of EGF in urine and plasma
Statistical Analysis
Acknowledgments

Results
- The effects of subcutaneously delivered EGF
- Stability of EGF in the colonic lumen
- The effects of luminally delivered EGF

Discussion

CHAPTER FOUR DEVELOPMENT AND VALIDATION OF AN ANIMAL MODEL OF COLITIS

Abstract

General Introduction

Carrageenan-induced colitis
  Introduction
  Method:
  - Acid hydrolysis of carrageenans
  - Animals and experimental design
  Comparison of the colitis-inducing properties of 3 types of carrageenan.
  Determination of the optimal dose of iota carrageenan required to induce colonic ulcerations
  - Statistical Analysis
  - Acknowledgments

Results
- Comparison of the colitis-inducing properties of 3 types of carrageenan.
- Determination of the optimal dose of iota carrageenan required to induce colonic ulcerations

Discussion

Acetic acid-induced colitis
  Introduction
  Methods
  - Experimental animals
  - Experimental trials
Exposure of the colonic serosa to acetic acid
Exposure of the colonic lumen to acetic acid
 exposure of the entire colonic lumen to acetic acid
 exposure of the distal colonic lumen to acetic acid
 exposure of a segment of the distal colonic lumen to acetic acid

- Analytical measurements
  Protein and DNA content of mucosal and muscularis samples
  Na+K+ ATPase activity
  Histological measurements

- Statistical Analysis
- Acknowledgments

Results

Exposure of the colonic serosa to acetic acid
Exposure of the colonic lumen to acetic acid
 exposure of the entire colonic lumen to acetic acid
 the effect of exposing the distal colonic lumen to acetic acid
time course recovery trial
an assessment of intestinal permeability using 51Cr-EDTA
 exposure of a 2cm segment of the distal colonic lumen to acetic acid

Discussion

CHAPTER FIVE

THE EFFECTS OF EXOGENOUS EGF ON THE ULCERATED RAT COLON

Abstract

Introduction

Methods

Animals and Experimental Design
  - Induction of colitis
  - Subcutaneous and Luminal EGF delivery
  - Stability of EGF in the colonic lumens of ulcerated rats

Analytical measurements
Measurement of EGF in urine and plasma
Statistical Analysis
Acknowledgments

Results

Plasma and urinary concentrations of EGF
Stability of EGF in the colonic lumen of ulcerated rats
Effect of 4 and 6 days treatment with EGF on repair of the damaged segment
Responses in the colonic segment adjacent to the ulceration
  a) Effects of acetic acid
  b) Effects of subcutaneous and terminally administered EGF
The effect of subcutaneous EGF infusion on the undamaged colonic segment
Discussion

CHAPTER SIX  FINAL DISCUSSION

APPENDIX A  HISTOLOGICAL METHODS

APPENDIX B  STATISTICS SUMMARY TABLES

BIBLIOGRAPHY
THEESIS ABSTRACT

Crohn's disease and ulcerative colitis are chronic forms of colitis for which the etiology is unknown and there is no known cure. Improvement in the existing treatments is still under clinical evaluation and there is a need for new effective therapies. The major emphasis of this thesis was to evaluate the potential therapeutic application of peptide growth factors, in particularly epidermal growth factor (EGF), in the treatment of colonic ulcerative conditions.

While the effect of exogenous EGF on ulcer healing, growth and maintenance of the upper gastrointestinal tract has been reported, its effect on ulcer healing in the colon has received little attention. In this thesis the responsiveness of the normal colon to EGF was assessed. A suitable model to assess effects of EGF on colonic ulcer repair was developed and used to assess the effects of exogenous EGF on the ulcerated colon.

The responsiveness of the normal adult colon to exogenous EGF delivered by continual subcutaneous infusion and by intraluminal bolus administration was measured. A 7 day continual subcutaneous infusion of EGF delivered by mini-osmotic pumps (200μg/kg/day), induced growth of the proximal colonic mucosa as shown by a significant increase in the mucosal wet weight, protein content and colonic circumference, as well as an increase in the number of cells per crypt and mucosal area above that of vehicle-treated control animals. Mucosal mass was also increased in the proximal colon where the wet weight and protein content was significantly elevated. Similar trends were observed in the distal colon although the magnitude of the response was lower than that seen in the proximal colon. In contrast, intraluminal EGF administration (1.6μg/kg/day) given as twice daily bolus injections, had no effect on colonic mucosal or muscularis growth in either the proximal or distal colon. The lack of effect of luminal EGF would not appear to be due to luminal degradation as radio-labelled EGF remained stable in the proximal and distal colonic
lumen for at least four hours. These findings suggest that the normal colon is responsive to exogenous EGF with systemic route being more effective than luminal delivery in eliciting a mitogenic response.

The assessment of new therapeutic therapies of colitis conditions in humans is necessarily limited in scope due to ethical constraints, therefore a suitable animal model for assessing the colonic ulcer healing effects of EGF was developed. Intraluminal application of acetic acid to a segment of the distal colon of adult rats, produced a distinct colonic lesion, which was reproducible, measurable and resolved over a 14 day period. A range of biochemical, physiological and histological markers of colonic damage were assed for their ability to quantitate the severity of the induced colonic lesion.

The same treatment regimes and doses used to assess the effect of exogenous EGF in the normal rat colon were used to measure its effect on the acetic acid damaged colon. EGF treatment was commenced at the time of the acid insult and assessed after 4 and 6 days. Neither luminal nor subcutaneous EGF administration enhanced re-epithelialization of the ulcerated colon after 4 or 6 days treatment, although a slight increase in mucosal hyperplasia was observed in the luminal EGF group in the region adjacent to the ulcerations where the crypt length was increased by 20% above that of vehicle-treated controls. The edema of the mucosa and submucosa layers in this region was significantly suppressed by subcutaneous EGF treatment, resulting in a reduction of the thickness of these layers by 20% and 42%, respectively, compared to vehicle-treated animals.

In conclusion, although EGF stimulates proliferation of the intact mucosa, it does not accelerate re-epithelialization of the acetic acid damaged colonic mucosa. Nevertheless, the reduction in edema and thickness of the submucosal and muscularis externa layers at the margins of the colonic lesion may confer some benefit to the damaged colon.