Colonic physiology in relation to the gut microflora
Declaration of Interests

Cereal Partners Worldwide
Nestlé & General Mills

Seagreens
Feed The Foundation Of Health

BBSRC
bioscience for the future

CPW SA

GREGGS

rb

Reckitt Benckiser

FMC BioPolymer
Know how. It works™

DRINC - DIET AND HEALTH RESEARCH INDUSTRY CLUB

ILSI SEA Conference: The Gut, Its Microbes and Health

Oct 8th-9th 2014
Presenter background
Talk structure

- Introduction to the colon
- Colonic physiology (and pathophysiology)

Themes

- The importance of the colon
- Potential for microbial influence
- Recent insights in the area
- What are we missing?
The Colon: From Banal to Brilliant

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\textbf{ABSTRACT}

The colon serves as the habitat for trillions of microbes, which it must maintain, regulate, and sequester. This is managed by what is termed the mucosal barrier. The mucosal barrier separates the gut flora from the host tissues; regulates the absorption of water, electrolytes, minerals, and vitamins; and facilitates host-flora interactions. Colonic homeostasis depends on a complex interaction between the microbiota and the mucosal epithelium, immune system, vasculature, stroma, and nervous system. Disruptions in the colonic microenvironment such as changes in microbial composition, epithelial cell function/proliferation/differentiation, mucus production/makeup, immune function, diet, motility, or blood flow may have substantial local and systemic consequences. Understanding the complex activities of the colon in health and disease is important in drug development, as xenobiotics can impact all segments of the colon. Direct and indirect effects of pharmaceuticals on intestinal function can produce adverse findings in laboratory animals and humans and can negatively impact drug development. This review will discuss normal colon homeostasis with examples, where applicable, of xenobiotics that disrupt normal function.
Henry Wheeler Shaw (1818 – 1885)

“A good reliable set of bowels is worth more to a man than any quantity of brains”.

www.collinsdictionary.com
Claude Bernard (1813 – 1878)

- Pioneering work on:
  - Scientific method in medicine
- Major breakthroughs in pancreatic/hepatic understanding
- Developed the idea of the “milieu interieur”

Human colonic function

- Removal of bodily waste
  - Toxic or indigestible material
  - Driven by smooth muscle action

- Fermentation
  - Indigestible carbohydrates (fibre and resistant starch)
  - Excess CHO, proteins and fats
  - Other dietary factors

- Salvage of nutrients
  - Water, low concentrations of minerals/other nutrients

- Protection
  - Innate and adaptive immunity
  - Self maintenance
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Colonic motility

Movement

Taenia coli

Haustra

Circular muscle
Normal bowel habit?

- 0 – 1 kg produced per day
- Three/day – 3/week
- Around 70% water
- pH neutral to acidic

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Saccharides
- Gases (CO$_2$, H$_2$, methane)
- Short-chain fatty acids (SCFA etc)
- Lactate

+ Proteins
- Ammonia and other nitrogenous compounds
- Sulphur-containing compounds

+ Glycerides
- Fatty acids (some conversion of PUFA to SFA)
SCFA are a metabolisable energy source for the body.

Therefore, dietary fibre provides energy to the body.

Most countries adopted a value of 2 kcal (8 kJ) per gram c. 2012.

### Other microbial metabolites

<table>
<thead>
<tr>
<th>Start product</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic acid</td>
<td>Caffeic acid</td>
</tr>
<tr>
<td>Naringen</td>
<td>Phlorogenic</td>
</tr>
<tr>
<td>Rutin</td>
<td>Quercetin</td>
</tr>
<tr>
<td>Catechins</td>
<td>Phenylvalerolactones</td>
</tr>
<tr>
<td>Phylloquinone</td>
<td>Metaquinone</td>
</tr>
<tr>
<td>Lignans</td>
<td>Active oesotrogens</td>
</tr>
</tbody>
</table>

Scalbert A et al., (2002) Biomedicine & Pharmacotherapy **56**
Metabolomic approach

(b) Highly ranked coffee exposure signals
Dihydrocaffeic acid conjugates

(c) Highly ranked oily fish exposure signals
Anserine

(d) Highly ranked tomato exposure signals
Hippuric acids derived from high content of hydroxycinnamates

PLUS
Dihydroxyphenylvalerolactone conjugates

Flavonoids derived from onions, fruits and wine co-consumed with tomato

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Colonic uptake mechanisms

### Fatty acid transport

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Tissue expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFAR1</td>
<td>C16-C22  β cell</td>
</tr>
<tr>
<td>FFAR2</td>
<td>C2-C4  Adipocytes</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>FFAR3</td>
<td>C3&gt;C4&gt;C2  Adipocytes</td>
</tr>
<tr>
<td>GPR84</td>
<td>C9-C14  Spleen</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>GPR120</td>
<td>C14-C22  Enteroendocrine cells</td>
</tr>
</tbody>
</table>

Vangaveti V et al., (2010) Therapeutic Advances in Endocrinology and Metabolism 1  
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Cell birth and cell death

- Birth
- Death

Epithelial insufficiency/mucosal damage

Epithelial homeostasis and health

Tumour growth
Colonic stem cells

- Located at base of crypt
  - Proliferation occurs in lower third

- Stem cell numbers per crypt tightly controlled

- Pluripotent and self-maintaining

Colonic mucosal immunity

Cell types

- Stem cells
- Absorptive cells
- Goblet cells
- Enteroendocrine cells
- Tuft cells
The mucus barrier

Mucus barrier

1. Removal with mucus flow
2. Diffusion through mucus layer
3. Penetration of the epithelial cells
### Factors in mucus

<table>
<thead>
<tr>
<th>Secretion</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>Opseninization of micro-organisms. Inactivation of bacterial toxins</td>
</tr>
<tr>
<td>IgM</td>
<td></td>
</tr>
</tbody>
</table>

**Antimicrobial peptides**

<table>
<thead>
<tr>
<th>Defensins</th>
<th>Bind to and destabilise bacterial membranes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathelicidins</td>
<td>Bacteriocidal against Gram- and Gram+. Chemolactic for white cells.</td>
</tr>
</tbody>
</table>

| Histatins                        | Antimicrobial via ROS generation, particularly against fungi              |
|----------------------------------|                                                                          |

| Lactoferrin                      | Deprives microbes of the essential nutrient Iron. Binds LPS and disrupts  |
|                                  | bacterial membrane. Has anti-biofilm activity.                           |

**Secretory proteinase inhibitors**

| SLP1                             | Inhibits serine-dependent proteinases.                                    |
|----------------------------------|                                                                          |
| Elafin                           | Bacteriocidal, but mode of antimicrobial action not fully characterised.  |

**Secreted enzymes**

| Lysozyme                         | Damage to bacterial cell walls                                           |
|----------------------------------|                                                                          |
| Secretory phospholipase A<sub>2</sub> | Microbiocidal via the generation of ROS and free radicals                |
| Lactoperoxidase/dual oxidase     |                                                                          |

**Antiadherence molecules**

| Surfactant                       | Inhibits mucosal adhesion. Enhances phagocytosis.                         |
|----------------------------------|                                                                          |
| Tamm Horsfall glycoprotein and related molecules | Inhibit mucosal adhesion. Stimulate PMNLs |
MUC2/MUC2 gene product

- Functional component = mucin
- “Bottle-brush” structure
- More negatively charged than gastric mucus
- Acts as a minimal barrier to absorption

Cysteine-rich domain
O-glycosylation regions
von Willebrand factor D-like domain
von Willebrand factor C-like domain
C-terminal domain

No Muc2 produced in knock-out mouse model

Mice developed tumours in the small and large intestine

Current model of interest for mechanisms of disease progression

Colonic mucus in health and disease

- In ulcerative colitis
  - Thinner, patchy layer
  - Change in glycosylation

- In colorectal cancer
  - Alteration in gene expression
  - MUC5AC expression

Pullan et al., (1994) Gut 35
Mucus as a niche?

Johansson MEV et al., (2008) PNAS 105
The crypt as a niche?

Pédron T *et al.* (2012) *mBio* 3
Where next?

The microflora
• Better characterize microbial niches
• Niche-niche interactions?
• How does microbiota interact with dietary factors?
• How does microbiota with the host?

Cause and effect?
• Animal studies
• Multiple controls in human studies
Acknowledgements

- Prof Jeff Pearson
- Prof Chris Seal
- Prof John Mathers
- Prof Peter Dettmar
- Dr Matt Wilcox
- Dr Peter Chater