Optimized fecal material for simulating bacterial drug degradation in the lower intestine

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In this presentation...

...lower intestine refers to
- Proximal Colon
- cecum
- distal ileum

populated by significant amount of bacteria
Bacterial degradation of orally administered therapeutic agents in the lower intestine: When is of interest?

- Absorption from the region is required for achieving or maintaining therapeutic effect, systemic or local
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A prodrug is administered and its conversion to the active form is expected to take place by the bacteria in the lumen of the lower intestine.

There is a risk of drug or drug-metabolite induced toxicity. The combination of sorivudine (an antiviral agent) with 5-fluorouracil (an antitumor agent) caused >15 deaths and numerous cases of serious side effects in the 1990s: sorivudine bacterial metabolite inhibits 5-fluorouracil metabolism in liver resulting in its abnormal accumulation in the blood and toxicity of 5-fluorouracil.
Outline

- How bacterial degradation of orally administered therapeutic agents in the lower intestine could be evaluated?
- Handling, storage conditions and reproducibility of fecal material
- Optimization of fecal material to reflect bacterial activity in the region of interest

Bacterial degradation of orally administered therapeutic agents in the lower intestine: How to evaluate?

Evaluation on drug degradation in the contents of the lower intestine

Measurement in humans by direct sampling from the lower intestine (colonoscopy) is associated with various difficulties
- medical supervision
- ethical issues
- sampling issues
  - tube’s positioning, limited number of samples, volume availability.
(Semi)continuous culture systems

- Long periods of incubation
- Part of the culture media is removed and replaced at intervals
- Addition of fermentable substrates (nutrient for bacteria) into the system

Simulator of the human intestinal microbial ecosystem (SHIME)

Five-stage reactor
The first two reactors are of the fill-and-draw principle to simulate different steps in food uptake and digestion.

The last three compartments simulate the large intestine. Upon inoculation with faecal microbiota, these reactors simulate the ascending, transverse and descending colon.

The model has been originally designed to study the metabolic fate of food over a period of several weeks.
Bacterial degradation of orally administered therapeutic agents in the lower intestine: How to evaluate?

MimiCol, Biostat®, TIM-2

<table>
<thead>
<tr>
<th>MimiCol</th>
<th>Biostat®</th>
<th>TIM-2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volume</strong></td>
<td>170 ± 40 mL ascending colon (Baidy et al., 1993)</td>
<td>150 mL</td>
</tr>
<tr>
<td><strong>Motility</strong></td>
<td>Pendulum movements, augmentations and fast mass transfer</td>
<td>6 rpm</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>7.4 to 6.2</td>
<td>6.2 ± 0.2 pH profile starting at pH 7.4</td>
</tr>
<tr>
<td><strong>Microbiota</strong></td>
<td>10^11 CFU/mL (Cummings and Macfarlane, 1991)</td>
<td>Standard microflora derived from a faecal sample of a healthy human volunteer</td>
</tr>
</tbody>
</table>


Static batch cultures
Caecal contents of animals (rats) or faeces (animal or human) are placed into a medium (saline or buffer solution)

- Suitable for short incubation periods
- Easy and flexible screening tool
- Estimation of bacterial degradation half-life of APIs (PBPK modelling)

### Points for consideration

- **Source:** animals vs. humans
- **Handling, storage conditions and reproducibility of bulk material**
- **Optimization of bacterial activity in the region of interest**
- The stool content in the final fecal material is crucial for the data to reflect degradation in lower intestine (literature data: ranges from 5 to 25%)

Bacterial degradation of orally administered therapeutic agents in the lower intestine: How to evaluate?

Static batch cultures
Caecal contents of animals (rats) or faeces (animal or human) are placed into a medium (saline or buffer solution)

<table>
<thead>
<tr>
<th>Species</th>
<th>Caecal contents (log/g)</th>
<th>Enzymes activities (mol/h per g caecal contents or faeces)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>10.8 ± 0.8</td>
<td>β-Glucosidase: 34.3 ± 5.5, β-Glucuronidase: 156.3 ± 28.8, Nitro reductase: 4.0 ± 0.7, Nitrate reductase: 3.9 ± 1.0</td>
</tr>
<tr>
<td>Mouse</td>
<td>10.2 ± 0.2</td>
<td>β-Glucosidase: 55.6 ± 22.0, β-Glucuronidase: 429 ± 4.6, Nitro reductase: 6.5 ± 0.7, Nitrate reductase: 1.8 ± 0.4</td>
</tr>
<tr>
<td>Hamster</td>
<td>10.4 ± 0.1</td>
<td>β-Glucosidase: 30.1 ± 2.3, β-Glucuronidase: 60.8 ± 14.8, Nitro reductase: 3.9 ± 0.6, Nitrate reductase: 1.7 ± 0.2</td>
</tr>
<tr>
<td>Guinea-pig</td>
<td>10.3 ± 0.1</td>
<td>β-Glucosidase: 8.4 ± 3.0, β-Glucuronidase: 113 ± 15, Nitro reductase: 0.4 ± 0.1, Nitrate reductase: 5.6 ± 1.8</td>
</tr>
<tr>
<td>Marmoset</td>
<td>10.8 ± 0.3</td>
<td>β-Glucosidase: 35.1 ± 8.0, β-Glucuronidase: 117 ± 5.5, Nitro reductase: 0.6 ± 0.2, Nitrate reductase: 1.9 ± 1.0</td>
</tr>
<tr>
<td>Human</td>
<td>11.3 ± 0.2</td>
<td>β-Glucosidase: 49.5 ± 8.1, β-Glucuronidase: 35.5 ± 19.9, Nitro reductase: 1.0 ± 0.2, Nitrate reductase: 8.0 ± 2.3</td>
</tr>
</tbody>
</table>

- The rat, guinea-pig and marmoset differed significantly from the human in three of the four enzymes
- The mouse and hamster differed significantly from the human two out of four enzymes

Sousa et al. Int. J. Pharm. 2008

Points for consideration
- Source: animals vs. humans
- Handling, storage conditions and reproducibility of bulk material
- Optimization of bacterial activity in the region of interest
  - The stool content in the final fecal material is crucial for the data to reflect degradation in lower intestine (literature data: ranges from 5 to 25% w/v)

Handling and storage of human fecal material

1. Freshly voided stools are collected in pre-weighted containers

2. Stools are transferred in an anaerobic workstation

3. Stools are weighted

4. Stools are diluted with normal saline (feces:saline 1:3.8 w/v, 25% w/v) and homogenized in a mixer

5. Material is sieved through a 0.35mm metal mesh to remove any heterogeneous fibrous material

6. Sieved homogenate is poured in sealed (screw cap) glass vials (to avoid multiple thawing freezing cycles)

7. pH of fecal material is measured

8. Sealed vials are stored at -70°C

Handling, storage and reproducibility of human fecal material

Enzyme activity is assessed indirectly by evaluating the degradation characteristics of 2 substrates of nitroreductase and azoreductase.

![Chemical structures of metronidazole and olsalazine with metabolites](image)

Metronidazole and olsalazine are chemically stable in the range of pH values that were achieved in human fecal material prepared and tested (5 < pH < 7)

Handling human fecal material

Optimization of incubation period of frozen fecal material on bacterial activity

Bacterial degradation experiments in previously frozen (-70 °C) human fecal material should be performed only after incubating the frozen material for about 2 h in the anaerobic workstation.
Handling human fecal material

Impact of second freeze-thaw cycle of fecal material on bacterial activity

Bacterial degradation experiments in previously frozen (-70 °C) human fecal material should be performed only after the first thawing of the material.

Storage of human fecal material

Impact of storage period of frozen fecal material on bacterial activity

During 48 months of storage at -70 °C bacterial activity remained unaltered.
Reproducibility of human fecal material
Evaluation of fecal material collected in two different occasions

Healthy adults (18-60 y)

Inclusion Criteria

• Within 20% of ideal body weight as determined by Metropolitan Life Tables.
• Willingness to abstain from smoking and drinking alcohol for at least 3 days before the day of stool collection.
• No treatment with antibiotics during the last 6 months before stool collection.
• No use of laxatives or cathartics for the last 7 days.
• Regular intestinal habits and stools consistency of type 2, 3, or 4, according to Bristol stool scale for at least a month before the day of stools collection.

Exclusion Criteria

• Exclusion of a specific food category from his/her diet (e.g., meat or dairy habits.)

No significant difference was observed
Optimization of the dilution of feces with normal saline

The stool content in the final fecal material is crucial for the data to reflect degradation in lower intestine
Literature data: dilution ranges from 5 to 25% w/v

Lack of justification of level of dilution of stools and the questionable clinical relevance of collected data may lead to
• rejection of potentially useful therapeutic agents (false negative decision) or
• selection of problematic compounds for further development (false positive decision)

Optimization of the dilution of feces with normal saline

For the optimization of dilution of the fecal material with normal saline to simulate ileal bacteria, the degradation profiles of model compounds were compared with their degradation profiles in the contents of the distal ileum
For the optimization of dilution of the fecal material with normal saline to simulate colonic bacteria, the degradation profiles of model compounds were compared with their degradation profiles in the contents of the proximal colon

Two issues
• Sampling from the lower intestine
• Population and Dosing conditions
Sampling from lower intestine...

... requires preparation of the distal colon without disturbing the physiological state of distal ileum/cecum/proximal colon at sampling time

One-time enema of 250ml of water appears sufficient to cleanse the left hemicolon

KULeuven | Lemmens et al. 2021

50h and 44h prior to colonoscopy 10mg bisacodyl [bis(p-acetoxyphenyl)-2-pyridylmethane] (Dulcolax®, mild laxative) is orally administered to prepare primarily the descending and sigmoid colon

NKUA | Diakidou et al. 2009

Bisacodyl for distal colon preparation?

After oral administration bisacodyl is rapidly converted to the active metabolite bis(p-hydroxyphenyl)-2-pyridylmethane (BHPM) and its action is initiated by activating protein kinase C releasing prostaglandin E2 and, thereby, inducing net fluid secretion (in vitro & human data)

Effects on the intraluminal physiology have been shown to be reversible (rat and human data).

Mucus secretion occurs only at BHPM concentrations of at least 1 μg/ml

Sodium and fluid secretion occurs at intracolonic BHPM concentrations of ≥5 μg/ml (rat data)

With the NKUA protocol for colon preparation, after overnight fasting and 5h after a glass of water, BHPM at proximal colon is ~ 0.5 μg/ml

Also, stools consistency and osmolality of contents at the proximal colon are used as supporting evidence

A useful methodology, especially when considering sensitive populations

Collection of contents of distal ileum, cecum, proximal colon

**Population**

**Healthy Adults**

**Dosing conditions**

**Oral drug absorption studies**

**Phase I - Fasted state**

- Semi-liquid food
- Start fasting
- Administration of water
- Colonoscopy

**Phase II - Fed state**

- Semi-liquid food
- Start fasting
- Administration of water
- Colonoscopy


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Bacterial degradation of *metronidazole* in the lower intestine

- **Distal Ileum**
- **Cecum**
- **Ascending Colon**

- **Fasted**
- **Fed**

- In the fed state, degradation, if any, does not increase much from distal ileum to proximal colon
- Bacterial degradation in cecum and ascending colon is more substantial in the fasted state

Bacterial degradation of olsalazine in the lower intestine

- In the fed state, degradation, if any, does not increase much from distal ileum to proximal colon
- Bacterial degradation in cecum and ascending colon is more substantial in the fasted state
- High inter-subject variability complicates the development of screening tools


Optimization of the dilution of feces with normal saline

Clinical importance of bacterial degradation of therapeutic agents in the lower intestine of adults ex vivo using adult fecal material was evaluated

- Based on
  - $P_{eff}$ human ileal and colonic permeability values of BCS highly permeable APIs
  - Residence times in the regions

- Assuming that
  - degradation is clinically important when $\geq$20% reduction in absorption from distal ileum or proximal colon occurs
  - degradation and absorption occurs according to 1st order kinetics

30min in distal ileum and 95min in proximal colon seem to be reasonable point estimates of maximum bacterial degradation half-lives in order bacterial degradation to be clinically important
Optimization of the dilution of feces with normal saline

30 min in distal ileum seems to be reasonable point estimate of maximum bacterial degradation half-lives in order bacterial degradation to be clinically important

Simulated ileal bacteria (SIB) consists of 5.5% (w/v) stools in normal saline


Optimization of the dilution of feces with normal saline

95 min in proximal colon seems to be reasonable point estimate of maximum bacterial degradation half-lives in order bacterial degradation to be clinically important

Simulated colonic bacteria (SCoB) consists of 8.3% (w/v) stools in normal saline

Evaluating the clinical importance of bacterial degradation of therapeutic agents in the lower intestine of adults using adult fecal material

Mean (SD) values for the bacterial degradation half-life (min) of model compounds tested in simulated intestinal bacteria (SIB) and in simulated colonic bacteria (SCoB)

<table>
<thead>
<tr>
<th></th>
<th>Simulated Ileal Bacteria (SIB)</th>
<th>Simulated Colonic Bacteria (SCoB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrendipine</td>
<td>26.9 ± 3.4</td>
<td>16.7 ± 1.6</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>73.2 ± 7.8</td>
<td>48.7 ± 2.7</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>13.42 ± 0.45</td>
<td>7.897 ± 0.090</td>
</tr>
</tbody>
</table>

Data in line with available literature data in humans

Vertzoni et al Eur J Pharm Sci 2018

Evaluating the clinical importance of bacterial degradation of therapeutic agents in the lower intestine of adults using adult fecal material

Is nitroreductase or azoreductase mediated degradation in the lower intestine likely to be clinically important for an orally administered therapeutic agent?

Currently, investigating the usefulness of SCoB in simulating sulfoxide reduction

Vertzoni et al Eur J Pharm Sci 2018
Key question: Can data be extrapolated to other populations?

To date, we have started considering this question for older people.

**Older people**
individuals older than 65 years old without any systemic diseases

Concluding Remarks

Data in SCoB are useful for evaluating whether bacterial azoreductase or nitroreductase activity in the proximal colon or in the distal ileum is likely to be clinically important for an orally administered therapeutic agent, provided that specific procedures are followed for:

- collecting feces
- preparing and storing fecal materials
- evaluating the degradation kinetics.

The usefulness of this approach worth further investigation in case of:

- therapeutic agents which are degraded by enzymes other than nitro or azo-reductases
- formulations targeting the lower intestine
- other populations (e.g. geriatric patients, paediatric populations, ...).
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Thank you for your attention!

I would be happy to answer questions!