Human Gastrointestinal Toxicity and Safety Assays: A New Model for Studying Drug Responses in Human Tissues
Biopta- Leaders in Human Fresh Tissue Research

- HQ and lab in Glasgow, UK
- Lab facility in Maryland
- Founded 2002
- First, and currently only, GLP compliant functional human tissue CRO
- Experts in human tissue research and procurement
- Pharma and Biotech customers worldwide
Sourcing of human fresh tissues: a 24/7 activity

- Receive ethically donated fresh tissues via two main routes:
  - Surgical residual tissues (mainly UK)
  - Non-transplantable organs (mainly USA)

- Facilities require to be open 24/7 to receive the tissues, dissect and conduct experiments – Laboratories placed strategically to minimise transport time

- Important to be ethically approved Research Tissue Bank and is able to deal with all aspects of sourcing and sharing of tissues with Sponsors and third party laboratories
Human Tissues Predict Clinical Safety and Efficacy

• **Functional data** produced in human *ex vivo* tissues

• Provide information to pharmaceutical and biotechnology companies about the *behaviour of their compounds in human relevant systems*

• **Efficacy**
  - Do the compounds work in human tissue?

• **Absorption/Metabolism**
  - Are the compounds permeable through human gut, skin, bronchi?

• **Safety Pharmacology**
  - Do compounds show adverse effects in human tissue?
  - Comparative studies with other species strengthens this capability
The GI tract is the organ most frequently subjected to side-effects. Commonly framed with respect to NSAIDs and chemotherapy, however, over 700 drugs are known to cause adverse GI effects. Perhaps seen as an unfortunate 'price to pay' for drug efficacy, but is an important cause of patient non-compliance; ADRs may cause 20-24% of all non-compliance. 

Survey of 100,000 patients

1. Chassany et al. (2000)
Predicting Gastrointestinal Toxicity

What forms of GI toxicity are of greatest concern?

• Bleeding/ulceration (mucositis/stomatitis)

• Disruption to barrier integrity, e.g. effects on the cytoskeleton

• Changes in secretory/absorptive functions

• Nausea and vomiting

• Indirect effects e.g. alterations to gut microflora leading to candidiasis; exposure to biliary excretion products
Which endpoints can we expect to model in human tissues?

From ICHS7A:

“Other organ systems (e.g., the renal or gastrointestinal system), the functions of which can be transiently disrupted by adverse pharmacodynamic effects without causing irreversible harm, are of less immediate investigative concern. Safety pharmacology evaluation of effects on these other systems may be of particular importance when considering factors such as the likely clinical trial or patient population (e.g. gastrointestinal tract in Crohn’s disease)”

“…c. Gastrointestinal System (2.8.2.3)
Effects of the test substance on the gastrointestinal system should be assessed. For example, gastric secretion, gastrointestinal injury potential, bile secretion, transit time in vivo, ileal contraction in vitro, gastric pH measurement, and pooling can be used.”
A range of gastrointestinal functions can be explored in human tissues.

**Tissue baths**
- **Motility.** Smooth muscle contractility (e.g. stomach, intestines or gallbladder)
- Nerve-muscle interaction

**Ussing chambers**
- Epithelial secretion, ion channel function/diarrhoea
- Epithelial barrier integrity

**Perfusion myographs**
- Vascular regulation
- Vascular leakage/permeability

**Ex vivo cultures/Precision-cut slices**
- GI injury potential
- Inflammatory processes (e.g. cytokines)
Assessing Gastrointestinal Motility in Human Tissues
Tissue Bath Set-Up

Example of an 8 bath set-up (Panlab)
AD Instruments Data Acquisition (Powerlab)

25 ml tissue bath containing physiological salt solution at constant 37 °C, gassed with 95% O₂/ 5% CO₂

Gastrointestinal tissue sample attached to a sensitive transducer that detects changes in muscle tension
Summary

Carbachol causes a concentration-dependent constriction in muscle strips orientated in both the circular and longitudinal plains.

At high concentrations carbachol can modulate protein kinases, and this could account for the relaxation response.
Do the models translate to the clinic?

Enhanced activity consistent with prokinetic effect \textit{in vivo}

Response to motilin absent in rats
Detecting Adverse Effects on GI Motility: Galantamine

- Acetylcholinesterase inhibitor used in treatment of Alzheimer’s disease (alkaloid isolated from snowdrops and daffodils)
- Patient compliance a major issue
- A number of pro-drugs being developed to avoid local GI effects
In vitro/ex vivo assays can be a useful support to in vivo models

The validation of an in vitro colonic motility assay as a biomarker for gastrointestinal adverse drug reactions

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• Potentially useful method for predicting ADRs

• Some gaps related to species differences from humans (e.g. motilin receptors)
Predicting changes in tissue integrity, secretory function and drug-metabolising enzymes in Ussing chambers.
Ussing Chambers: Multiple GI Tox Endpoints

Voltage and current electrodes: electrical parameters can be measured to monitor ion flow, cell integrity etc.

Tissue forms a barrier between right and left chambers

Endpoints related to GI toxicity:

- **Tissue integrity/tight junctions**- transepithelial resistance
- Diarrhoea/constipation- **secretory mechanisms** controlled via ion channels
- Drug-metabolising enzymes and drug transporters- toxicity related to production of **toxic metabolites** and their active efflux (potential for **drug-drug interactions**)

human tissue experts
Human Test Systems

- Ussing system experiments most often conducted using mucosal layers isolated from two areas: respiratory tract and gastrointestinal tract
  - Mucosal layer of trachea or primary bronchus
  - Mucosal layer of stomach or small or large intestine
  - Measure changes in transepithelial resistance, total short-circuit current or movement of specific ions
  - Measure drug absorption, metabolism, transporter function
Permeability
Human Duodenum Reference Compound $P_{app}$
Mean values + SD

$n=$ number of patients, $P_{app}$ is low for non-permeable compounds and high for highly permeable compounds.
Does human *in vitro* data translate to the clinic?
An increase in the short circuit current is observed following the addition of cholera toxin corresponding to the increased efflux of ions to the apical side of the tissue. The addition of bumetanide causes a decrease in the short circuit current since bumetanide blocks the Na-K chloride transporter and inhibits the efflux.
STc and Linaclotide induce transepithelial ion current in human ascending colon

Further mechanistic understanding by combining this with radiolabels

Decreased absorption of $^{22}\text{Na}$ in response to GC-C agonists
Human GI tract has a physiologically-relevant balance of DMEs and transporters

- Reactive metabolites of importance in many drug-mediated side-effects
- Gradients of expression along the length of the GI tract (CYP high in small intestine, decreases towards colon; opp for Pgp and MRP3).
- Together, the expression profiles determine the exposure of the enterocytes to toxicants
Mediator Release in Human Colon: GLP-1 Release Assay

Legend:
- 2-OG = 2-oleoyl glycerol (GPR119 endogenous ligand)
- X1 = client compound (undisclosed target)

Biopta was sponsored by a client to determine whether GLP-1 release can be measured from human ex vivo colon.
Human Gastrointestinal Explant Cultures and Precision-Cut Intestinal Slices
Tissue Explants and Precision-Cut Slices: Maximising Throughput

**Tissue Explants**

- Experience with tissue culture models of 2-5 mm size explants;
- 20-30 explants per patient
- Lower throughput but highly valuable models of disease processes

**Tissue Slices**

- Precision cut slices
- 10’s-100’s of slices per patient
- 100-250 μm slices from diseased human tissue that can supply biology to HCS systems

HCS – high content screening
Multiple full thickness gut mucosal samples dissected from fresh tissue sample
Colon explant histopathology maintained over 24 hour culture period

0 hours: Good crypt morphology, intact lamina propria and muscularis mucosa

24 hours: Maintained crypt morphology and muscularis mucosa. Slight degradation of epithelial structure but generally intact
Is healthy margin, surgical resection tissue “normal” and if so, does culturing change this?

Principal component analysis of hCellMarkerPlex gene expression profiles

Healthy colon (blue circles)
Surgical residual 0 hour culture (blue triangles)
Surgical residual 1 hour culture (turquoise triangles)
Surgical residual 2 hour culture (light blue triangles)
Surgical residual 4 hour culture (grey triangles)
Surgical residual 14 hour (open triangles)
Adenoma colon (green circles)
Carcinoma tissues (red circles).

Surgical residual biopsies show a normal healthy colon gene expression profile which is distinct from carcinoma and adenoma tissue. This is unaffected by the culturing process.
Diseased colon explant histopathology

**Crohn’s Colon:** Shortened and widespread crypts, increased lamina propria cellularity and granuloma present

**Ulcerative Colitis:** Crypt destruction, widespread mucosal erosion and severely thickened muscularis mucosa
Distinct differences in cytokine profiles can be observed between each disease state.

Modulation of these profiles can be assessed with test articles.
Cytokine Profile in Crohn's Tissue

The cytokine profile observed in Crohn's tissue followed the expected Th1/Th17 profile with elevated levels of various standard markers of this phenotype such as IL-17A, IFN\(\gamma\), TNF\(\alpha\) and IL-12.
The cytokine profile observed in ulcerative colitis tissue followed an atypical Th2 profile with elevated levels of pro-inflammatory cytokines such as IL-1beta and IL-10 but relatively unchanged levels of the classical Th2 markers IL-4 and IL-5.
Effect of Fluticasone Proprionate (Corticosteroid) on Explant Cultures of UC Tissues

- Cytokine profiles following 24 hour culture period; n = 3 Ulcerative Colitis patients.
- FP concentration = 0.1 µg/mL
Precision-Cut Tissue Slices

**Steps:**
1. Whole diseased organs or tissues
2. Tissue stabilised with agarose
3. Cores taken through tissue
4. Slices cut
5. Slices suspended on a mesh in culture medium
Precision-Cut Intestinal Slices (PCIS) and the prediction of GI toxicity

- PCIS of growing interest as a way to model xenobiotic toxicity, metabolism and transport

- Initial focus has been on drug-metabolising enzymes and related toxicity

- Further work required to establish a range of toxicity biomarkers and testing against a panel of compounds

**Review Article**

Evaluation of the intestinal toxicity and transport of xenobiotics utilizing precision-cut slices

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Diclofenac toxicity: apparent differences in mechanism of toxicity in rats and humans

- Diclofenac associated with high prevalence of GI side effects
- *In vivo* studies in rodents suggest tox related to reactive metabolites.
- PCIS from human jejunum used as *ex vivo* allowed detailed investigations of potential mechanisms of toxicity in human GI tract.
- By studying the human SI directly, the contribution of GI-generated metabolites and any direct effects of the parent compound could be assessed.
- Study found that diclofenac can cause direct toxicity

Archives of Toxicology, April 2014.
What does the future hold?

- Evidence that GI toxicity has a significant impact on patient compliance
- Access to human tissues is improving
- Models to address many tox endpoints already exists
- Miniaturisation is increasing throughput and reducing cost
- Exciting times for human tissue research

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