Absorption - Models and Methods

Dr.K.Latha
associate Professor
Department of pharmaceutics

- Drugs---- therapeutic response
- Dosage form----- release studies, therapy
- Factors affecting ----- optimize
- Kinetics-------- how and at what rate ADME process occurs
- Biological barriers----epithelial, endothelial, elimination
- Biological barriers----*in situ*, *in vitro*

- Epithelial barriers—intestinal, vaginal, rectal, oral, nasal & respiratory, ocular, dermal.
- Endothelial barriers– blood brain barrier.
- Elimination– liver and kidney
- Remove drug from systemic circulation and secretion of drugs into bile and urine—reduced blood level and efficacy

Desirable characteristics

- Physiologically reflective
- Monolayer and mucus
- Biochemical---

Transporters (peptide transporters)

Efflux system (p- glycoproteins)

Metabolic enzymes (amino peptidases) at the same level.

- Low to moderate level of technical expertise to set up and maintain.

- Large no. of studies rapidly and inexpensively.
- Ideal model--- maintained using simple media (Hank's balanced salt solution)
- Std. methods such as HPLC, LC-MS to determine the disposition.
- Satisfy- *in vitro, in situ* studies.
- Limitation- over interpretation of data.

Characterization

- Duplicating the data
- Vary with model
- Perfused organs--- viability of cells during transportation, metabolism.

Same physical biochemical barrier properties.

Physical damage---

1.detected by light microscopy, SEM

2.radilabbeled markers--- inulin, mannitol

3. transepithelial electrical resistance value

4. activity of transporters or enzymes.

CELL CULTURE

- Columnar shape, functional complexes, well defined brush border with microvilli.
- Histochemical localization studies.
- Distribution of alkaline phosphatase on their luminal side.
- Impermeable hydrophilic markers (inulin).
- Function integrity by endogenous transporters, efflux systems, enzyme activity.
- Viability of cells---

1. measured by actively transported solutes (glucose, amino acids)
2. chloride secretary rate.

Applications

- Estimation of permeability
- Elucidation transport path ways—para, transcellular
- Transport mechanisms– passive, carrier mediated.
- Determination of structure-transport relationship.
- Determination of protein binding (influence of components)

- Determination of optimal physicochemical properties.(size, charge, lipophilicity, hydrogen bonding)

- Structure-transport relationships for efflux systems.

- Enhance membrane permeability – 1.chemical strategies (prodrugs)
  2.formulation (adjuvants)

- Assessment of potential toxic effects.

- Elucidation of potential pathways of drug metabolism/elimination.

Evaluating methods

- Successful design----

1.stable to chemical and enzymatic degradation

2. able to transverse to portal circulation and enter in to systemic as intact form.

3. needs less compound

4. easier method

5.avoids complicated surgery.

- More rapid, reduce animal usage.
- Analytically more simple.

Methods

- Buffers: Na+--141, K+--- 5, Ca2+---1.2, Mg2+---1.2, Cl- ---122, HCO3- ---25, H2PO4- ---0.4, HPO4- ---1.6. Having pH of 7.4, gassed with 5% Co2 in O2.

Alternative buffer – pH or ion-dependence on permeability, stability of drugs.

- Tissues---
- bovine colon (stripped)

Bull frog small intestine (stripped & unstripped)

- Canine---- duodenum, jejunum, ileum, colon (unstripped)
- Chicken-- same as above
- Frog colon (unstripped)
- Guinea pig ileum (unstripped), colon (stripped)
- Human --- cecum, proximal colon, transverse colon, sigmoid colon, ileum, jejunum (stripped)
- Monkey --- deodenum, jejunum,ileum,colon (unstripped)

- Rabbit-- deodenum, jejunum (stripped) cecum, proximal colon, distal colon (stripped)
- Rat --- jejunum, ileum (unstripped)

Equipment: Ussing chamber

1. Thermostatic reservoir, temp bath solution for oxygenation and circulation of buffer
2. Voltage clamps for measuring potential difference.
3. Short circuit current- for electrical driving force on transport/permeability of molecules.
4. Gas for oxygenation to reduce tissue evaporation.

5.circulation system---- to reduce the thickness of unstirred water layer (UWL).

6. modified ussing set up is with water jacketed reservoirs.

Tissue preparation:

Unstripped: prepared by opening the intestine along the mesenteric border and placing pieces of the intact mucosa.

Stripped: prepared by opening the intestine along the mesenteric border, remove the circular and longitudinal muscle layers -----muscle deficient tissue.

Stripped are preferred due to close resemble to *in vivo* situation.

- Small intestine:
- New Zealand white male rabbit--wt 2-3 kg, killed by cervical dislocation.
- Duodenum-10 cm, jejunum-20 cm, ileum-15 cm, removed quickly, cut along mesenteric border and rinsed with ice cold bicarbonate buffer. Two muscle layers are removed field&co-workers technique.
- Tissue is placed serosal surface facing up on plexiglas plate and kept moist with bicarbonate buffer.
- With scalpel blade, a transverse incision is made through the circular and longitudinal muscle layer, removed with forceps.

- Stripped tissue consists of epithelium, underlying lamina propria and muscularis mucosae.

- Colonic tissues: same as above for proximal colon

- Distal colon segments are stripped and placed mucosal surface up on plexiglas plate kept moist with bicarbonate buffer.

- Tissue is placed on pins at one end, streched lengthwise and held with a glass microscope slide. Second glass microscope slide is used to gently scrape the muscle layer from the mucosa.

Transport studies

- Electrical measurements:
- Tissues are placed between half chambers and perfused separately on either side with 5-12 ml of bicarbonate buffer.
- Chambers are equipped with ports for measurement of potential difference.
- If the chamber is connected to calomel half cells through salt/agar bridges.

- Agar bridges should be filled with buffer that is employed to studies.
- Preparation of agar bridge:

Polyethylene tubing is filled with agar (4.5 g) in buffer (90 ml)--- slow boiling with stirring.

Upon cooling tubings cut to 6 in length, at distal ends ports are provided for current passing bridges and electrodes with voltage clamp.

With current/voltage clamp, PD can be clamped to zero or any desired value.

current required to nullify PD is short circuit current *Isc* --- is measure of tissue viability – change in this is resulting from addition of an absorptive or secretary stimulus (glucose or prostaglandin)

Transepithelial conductance (Gt), or resistance (Rt) can be determined by employing ohm's law.

$$PD = I.Rt$$

$$PD = I/Gt$$

In leaky epithelia--- significant increase in permeability can occur with no measurable changes in Rt.

Experimental protocol

- Tissue chambers are connected to perfusion reservoirs
- Equilibrate for 30-60 min to establish ion-transport process.
- Tissues are bathing with bicarbonate buffer with 10Mm mannitol, 8Mm glucose.
- Serosal bathing contains 2Mm mannitol. After equilibrium period, molecule to be studied is added to mucosal or seroral bathing solution.

- At definite time intervals >15min sample of 0.5-1.0 ml is removed and replaced with fresh soln. to maintain constant volume, ionic composition and hydrostatic pressure.

- Sampling is done from donor bathing solution.

- For transmural flux is determined on pair of tissues.

- Tissues are paired on basis of electrical resistance differed less than 25% . If not done mannitol permeability is used for selection criterion.

calculations

- Papp=(Vr.dCr)/A.Co.dt)

Vr= vol.of receiver chamber.

A= exposed tissue surface area

Co= conc. of donor chamber

dCr/dt= change in conc. of receiver bathing solution with time.