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METHODS FOR ASSESSMENT OF GASTROINTESTINAL DRUG ACTIONS

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Many types of drugs affect functions of the gastrointestinal tract. Investigators may be interested in discovery or pharmacological characterization of drugs as therapeutic agents intended for treatment of gastrointestinal disorders or in identification of gastrointestinal side effects of drugs intended for non-gastrointestinal indications. Examples of drug categories often associated with significant gastrointestinal side effects include cardiovascular drugs, antibiotics (erythromycin in particular), anti-inflammatory drugs, antiemetics, analgesics (especially opiates), antihistamines, antidepressants, and antipsychotics. Whether the objective is development of gastrointestinal therapeutic agents or evaluation of gastrointestinal side effects, appropriate laboratory models for experimentation are essential.

SELECTION OF MODELS

Drug discovery programs may be directed at a number of worthwhile therapeutic targets to address inadequately treated gastrointestinal diseases. These targets may include analgesics for visceral pain, antidiarrheal agents, anti-inflammatory drugs for inflammatory bowel disease (IBD), antiulcer or antiesophagitis drugs, and drugs for treatment of motility disorders, such as prokinetic drugs for gastroparesis or ileus, or for treatment of functional disorders, such as irritable bowel syndrome (IBS) or nonulcer dyspepsia. Selection of the specific model to be employed must be based both on pharmacologic considerations and on intended use of the model.

Pharmacologic consideration include the specific therapeutic target, sites of drug action, mechanism of action, and method of drug delivery. For example, if the therapeutic target is

treatment of esophagitis, model selection will depend on whether the intended mechanism of action is protection of the esophageal mucosa, improvement of esophageal clearance, increase in tone of the lower esophageal sphincter, neutralization of gastric acid, or inhibition of gastric acid secretion. Certain of these mechanisms could involve different sites of action (esophageal mucosa, esophageal smooth muscle or intrinsic motor nerves, gastric parietal cells) and different drug delivery strategies (local intraluminal actions or actions after systemic absorption).

Once decisions have been made about the therapeutic target, mechanism of action and sites of action, another set of consideration will be required, namely, whether the model should be used primarily for purposes of screening large numbers of chemical compounds or will be used to provide definite information about sites and mechanism of action. For example, if the therapeutic target is improved gastric emptying for treatment of gastroparesis, a simple screen in intact mice could be used, or consideration could be given to a more elaborate rat model for examination of small intestinal propulsion and colonic propulsion in addition to gastric emptying, a model of alpha$_2$ adrenergic agonist-induced gastroparesis (Gullikson et al., 1991), or a model that separately defines gastric emptying of both solids and liquids. In general, models intended for rapid screening of compounds should be simple, fast and inexpensive. Whenever possible, in vitro models should be employed if the desired mechanism can be measured quantitatively in vitro. Definitive models may be more elaborate, and allow direct or indirect measurement of the precise endpoint required for the therapeutic target.

In drug discovery programs, sequential models may be necessary. For example, if the desired mechanism is agonist action at a specific type or subtype of receptor with a therapeutic target of improving gastric emptying, the medicinal chemistry program may best be supported by an in vitro ligand binding assay with membrane preparations or with cells expressing the particular cloned receptor of interest. The ligand binding assay can provide rapid feedback to the design chemists for structure-activity relationships. It is usually necessary to examine efficacy as well as receptor affinity, so a second sequential assay, such as an in vitro bioassay with cells (measure effects of compounds on second messenger chemicals) or tissue (strips of gastric antrum) may be suitable. Once lead compounds are identified, they may be examined in a simple mouse model of gastric emptying (gavage with a nonabsorbable marker), a more precise rat model, or a dog model that permits separate measurement of solids and liquids

(Gullikson et al, 1993). The sequential models are selected as necessary for each stage of the drug discovery and development process.

Appropriate selection of species is necessary for in vivo models. Antinociception (analgesia) is often best studied in mice, rats and monkeys, because prior experience has shown good correlations between analgesic activity in these species and activity in humans. Emesis and antiemetic drugs must be evaluated in species with complete emetic mechanisms, such as ferrets, cats and dogs. Studies of motility, propulsion or mucosal transport may be conducted with rodents or other mammalian species. If intracerebroventricular (i.c.v.) or intrathecal (i.t.) injections are necessary for site of action studies, mice and rats are more convenient than cats, dogs or monkeys (Porreca et al., 1984). For some drugs, such as opioids, species-dependent effects on patterns of gastrointestinal motility are well established (Pruitt et al., 1974).

SPECIFIC MODELS

Gastrointestinal Propulsion

Propulsion or flow is measured by transit of marker from one site of the gastrointestinal tract to another. Gastric emptying, small intestinal propulsion or colonic propulsion can be measured. In each case, the basic principle is the same: assessment of movement of a nonabsorbable marker. Gastric emptying is the easiest to measure (Burks et al., 1985). A precise amount of an easily quantitated nonabsorbable marker is placed in a convenient volume (0.1-0.2 ml) directly into the mouse or rat stomach by use of a gavage needle, time (usually 20-30 min) for fractional emptying is allowed to elapse, then the animal is killed, the stomach removed, and the amount of marker remaining in the stomach is measured quantitatively for calculation of the fraction emptied. The most convenient marker for study of gastric emptying in rats or mice is ^{51}Cr (given as a solution of $Na_2\,^{51}CrO_4$ in saline). The amount of radioactivity remaining in the stomach can be determined by gamma counting of the entire stomach with its contents. ^{14}C-Polyethylene glycol can also be used, but the gastric contents must be removed and mixed in a suitable cocktail for liquid scintillation counting. Similarly, phenol red dye can be used, with the concentration in the gastric contents determined spectrophotometrically.

Small intestinal transit is best evaluated by bulk flow of a nonabsorbable marker and measurement of regional distribution within the small bowel, the "geometric center" method (Miller et al., 1981). Measurement of the distance traveled by India ink or charcoal is not satisfactory because only the leading edge of marker movement can be determined. The geometric center of ^{51}Cr distribution gives a precise numerical value for total propulsion within the lumen. In the geometric center method, rats or mice receive approximately 100,000 counts/min of ^{51}Cr in 0.1 or 0.2 ml of saline by gavage or by instillation through a previously implanted (in rats) duodenal catheter (to avoid vagaries resulting from drug effects on the rate of gastric emptying). After 25-35 min, the animal is killed and the stomach and small intestine are removed. The small intestine is divided into 10 segments of equal length by means of a template, and each segment is placed in a tube for gamma counting. The distribution of radioactivity in the 10 segments allows calculation of the geometric center of transit:

$$\text{Geometric center} = \Sigma \frac{(\text{counts/min in each segment})\ (\text{segment number})}{\text{total counts in intestine}}$$

The geometric center method is also useful for evaluating transit in the colon in rats (Williams et al., 1987). For measurement of colonic transit, an indwelling catheter for isotope instillation is placed in the proximal colon in a previous surgery.

An alternative method for assessment of colonic propulsion of solids is measurement of the time required for expulsion of small (1 mm diameter) glass beads inserted per rectum into the colon of mice (Porreca and Burks, 1986).

Measurement of propulsion in larger species, such as unanesthetized dogs, is much more complicated than in mice and rats. Propulsion in large species is most often accomplished by oral feeding of ^{99m}Tc sulfur colloid and movement of the label is followed with scintigraphy involving use of a gamma camera.

Gastrointestinal Motility

Methods for assessing contractions in vivo differ in terms of the specific endpoint actually

measured. Use of monopolar or bipolar recording electrodes fixed on the serosal surface of the intestine allows measurement of the incidence of contractions, expressed as spike potentials superimposed on the regular pattern of myoelectric slow waves (Sarna et al., 1981). Because of the relative simplicity of myoelectric recordings, an array of 6-12 electrodes can be placed along the stomach, small intestine and colon for identification of overall patterns of contractile activity. The disadvantage of myoelectric recordings is that the amplitudes of contractions cannot be directly assessed and the underlying intraluminal pressure associated with contractions is not known.

Extraluminal force transducers, attached by sutures to the serosal surface of the stomach or intestine, allow direct measurement of amplitudes of contractions (Itoh et al., 1981). An array of 6-12 strain gages can be implanted to allow identification of migrating motor complexes and regional differences in contractile activity (Itoh and Sekiguchi, 1983). However, extraluminal force transducers do not directly measure pressure within the lumen of the intestine.

Intraluminal pressure in unanesthetized rats can be measured conveniently by use of indwelling, open-tip small-diameter catheters perfused by means of a low-compliance pneumohydraulic pump with water or saline (Arndorfer et al., 1977; Galligan and Burks, 1983). Outflow resistance from the luminal catheter tips is measured by pressure transducers. A low volume of flow is necessary to avoid overwhelming the absorptive capacity of the intestine, thus leading to accumulation of fluid in the intestinal lumen. The advantage of this technique is that it allows direct measurement of intraluminal pressure, the major determinant of propulsion in the intestine. The disadvantages are that, for practical reasons, only 2-4 catheters can be implanted in an individual rat and that excessive movement of the animal can result in artifacts.

In anesthetized animals, such as dogs, intraluminal balloons attached to pressure transducers faithfully record intraluminal pressure (Pruitt et al., 1974; Burks et al., 1974). However, balloons are not suitable for chronic implantation in animals because they tend to impede flow of intestinal contents.

An ex vivo preparation of dog, cat or monkey intestine can be used for studies of drug effects directly on nerves, muscle or mucosa of the small bowel (Burks et al., 1974). The

advantage of the preparation is that it provides a useful model for motility studies in the essentially unanesthetized small intestine, yet can be prepared from acutely anesthetized animals. An mesenteric artery supplying a short (5-10 cm) segment of small intestine is cannulated for perfusion with Kreb's solution, ligated on both sides of the perfused segment, and excised for arterial perfusion ex vivo. Intraluminal pressure is monitored from a balloon placed in the lumen, and drugs are administered as intraarterial boluses or dissolved in the perfusion solution. This preparation has been especially useful for studies of opioids and other excitatory neuropeptides (Davis et al., 1991).

Mucosal Transport

Unidirectional or net transport of electrolytes across the intestinal mucosa is best measured in vitro in an Ussing chamber (Sheldon et al., 1990). Changes in short-circuit current necessary to maintain transmucosal potential difference are indicative of net ion flux from mucosal to serosal or serosal to mucosal surfaces. Unidirectional flux of chloride, sodium, potassium or calcium can be assessed by passage of radioisotopes of the ions across the mucosa.

Mucosal transport is more difficult to measure in vivo in unanesthetized animals. The most useful measure of mucosal secretion of fluid into the lumen is provided by evaluation of enteropooling (Farmer and Burks, 1991). In the enteropooling model, net fluid secretion into the intestinal lumen is measured by removal of the small bowel (mice, hamsters or rats) and expression of the fluid content. The difference in weight of the intestine before and after removal of luminal fluid is indicative of net secretion. Secretion can be stimulated by administration of prostaglandin E_2 or another secretagogue.

Another approach to evaluation of mucosal secretion in mice and rats is by inducing diarrhea, which can be measured quantitatively by weight of fecal excretion, body weight loss of animals, and time after administration of secretagogue to appearance of diarrhea (Shook et al., 1989). In larger animals, such as dogs, polyethylene glycol solution can be perfused through the intestinal lumen from an inlet catheter to a collection catheter, and the concentration of polyethylene glycol in the effluent in comparison with the inflow concentration reveals whether water was absorbed from the lumen or secreted into the lumen. This technique is best

carried out in anesthetized animals.

Gastric Acid Secretion

In anesthetized rats, gastric secretion is readily measured by ligation of the pylorus, to prevent flow of gastric juice into the duodenum, and sometimes by ligation of the lower esophagus, to prevent reflux of gastric juice into the esophagus. Esophageal ligation, however, damages the vagal nerve trunks entering the stomach and may thereby alter the pharmacological effects of test drugs. Either basal or stimulated (by histamine or pentagastrin) secretion is measured by collection of the gastric fluid for determination of volume and total acidity by titration (Fox and Burks, 1988).

Gastric secretion is generally assessed in unanesthetized rats, cats and dogs by use of gastric fistulas, with or without creation of Heidenhain or Pavlov pouches, with gastric juice collected periodically (every 10-20 min) during an experiment (Bado et al., 1991). The fistulas are closed between experiments to allow normal digestion and these preparations can be used for extended periods of time (weeks, months or years).

Inflammatory Bowel Disease

The most common experimental model for studies of gastrointestinal anti-inflammatory agents is mucosal inflammation brought about by luminal exposure to a proinflammatory agent, such as trinitrobenzenesulfonic acid (TNBS). The degree of inflammation is assessed by measurement of volume of fluid secreted into the lumen, amount of protein secreted into the lumen, and by the level of activity of tissue myeloperoxidase (Miller et al., 1993). Anti-inflammatory drugs can be administered prior to exposure of the mucosa to TNBS or after the inflammatory condition has been established.

Another useful model of inflammation takes advantage of the ability of the intraepithelial nematode, Trichinella spiralis, to produce inflammation in the jejunal mucosa and submucosa (Swain et al., 1991). Primary infections with Trichinella spiralis can be induced to create chronic inflammation of the proximal small bowel.

Functional Motility Disorders

Disordered motility in characteristic of irritable bowel syndrome (IBS) and possibly other functional disorders which are induced or exacerbated by stress. The most useful models for IBS use stressors to induce disordered motility and propulsion. The first stress model in rats that reproduces many of the motility changes characteristic of IBS in humans was the wrap restraint model (Williams et al., 1987, 1988). In the wrap restraint model, paper tape is wrapped around the foreshoulders and thorax of rats in a manner that modestly restricts movement of the forelimbs to reduce grooming of the ears and head, but without limiting ability to grasp and eat food. The wrap restraint is associated with elevated plasma levels of adrenocorticotropin (ACTH) and B-endorphin, biological markers of the stress response. Wrap restraint is also associated with altered patterns of small intestinal, cecal and colonic motility, decreased propulsion in the small intestine, increased propulsion in the colon, and increased fecal output (Williams et al., 1987, 1988; Burks, 1991).

A similar rat model uses aversive electrical footshock to condition fear in animals placed in the box in which they previously received unavoidable footshock (Gue' et al., 1991). The conditioned fear response is thought to model mental stress and results in changes in motility and propulsion essentially identical to those associated with wrap restraint stress.

Visceral Sensory Functions

Activity of sensory nerves in visceral afferent pathways can be measured functionally, behaviorally, or electrophysiologically. Only electrophysiological recordings provide direct measures of sensory nerve activity. In general, two approaches have been employed for direct recording of sensory nerve impulses: placement of recording electrodes on the peripheral ends of sectioned or ligated vagus nerves, which carry most physiological afferent signals from the gastrointestinal system, or by recording from depth electrodes placed in the brainstem nucleus and tractus solitarius (Barber et al., 1987, 1994; Barber and Burks, 1987). Afferent neurons that travel over the vagus have obligatory synapses in the nucleus of the tractus solitarius (NTS) and extracellular recordings from NTS neurons provide accurate measures of excitatory and inhibitory input from the upper gastrointestinal tract to the NTS.

Table 1. Useful in vivo methods for screening or definitive studies of gastrointestinal drugs.

Parameter	Screening Tests	Definitive tests
G.I. propulsion	Mouse transit	Rat regional transit
G.I. motility		Rat motility in vivo
Mucosal transport	Mouse enteropooling	
Gastric secretion	Anesthetized rat	Chronic gastric fistula rat
IBD	Hamster TNBS	Guinea pig or rabbit TNBS
IBS		Rat stress model
Visceral sensory activity		NTS recordings

G.I. = gastrointestinal
IBD = inflammatory bowel disease
IBS = irritable bowel syndrome
TNBS = trinitrobenzenesulfonic acid
NTS = nucleus tractus solitarius

CONCLUSIONS

Many useful types of approaches may be employed to evaluate the effects of experimental or established drugs on functions of the gastrointestinal tract. Some models are suitable for screening of compounds for desired profiles of pharmacologic activity, other models are more suitable for definitive mechanistic investigations. Several potentially suitable models are listed in Table 1. In many cases, models may be used sequentially, one as an initial screen and another for more mechanistic evaluation of drug candidates.

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