Screening of drugs acting on G.I.T

I. Study of antispasmodic activity.

II. Study of anti-ulcer effect.

III. Study of purgative action.

IV. Study of anti-emetic action.



Antispasmodic (spasmolytic)

Drugs which has the ability to reduce muscle tone and are used in a variety of conditions characterized by smooth muscle spasm such as:

- biliary colic
- intestinal and renal colic

e.g.: atropine

I. Screening of antispasmodic activity

- Antispasmodics can be assessed by their inhibitory action on smooth muscle preparations stimulated by contraction by Ach.
- Possession of local anesthetic activity and absence of strong mydriatic activity are additional advantages in antispasmodic drugs and these should be tested for.

Mebeverine hydrochloride, a spasmolytic agent on GIT smooth muscles, have a local anesthetic effect.

In-Vitro:

- Isolated rat ileum/ guinea pig ileum/isolated rat colon/, duodenum.
- (2 cm piece). Mounted in organ bath (Tyrode; 37°C, O2)
- Agonists such as acetylcholine (10⁻⁴M) and BaCl2 exerted almost identical spasmogenic effect. (standard stimulant of gastrointestinal smooth muscle).
- Atropine 10⁻⁶M (standard antispasmodic)
- Compare amplitude of contractions (mm).

<u>In-Vivo:</u>

- Topically applied KCl to rat urinary bladder (in-vivo).
- This model was first described by Postius and Szelenyi in 1983 (<u>J Pharmacol Methods.</u> 1983 Feb;9(1):53-61)
- Test drug may be given IV.





Figure 1. Spasmolytic effect of 10^{-6} M atropine sulfate and different concentrations of hops extracts on 10^{-4} M acetylcholine-induced contractions in isolated rat's ileum (n=6). * indicates the significant difference (p<0.05) as compared with the Achinduced contraction according to the one way ANOVA fallowed by Tukey's post-hoc test.



Peptic Ulcer

A condition that describes the discontinuity in the entire thickness of the gastric and duodenal mucosa.



Peptic Ulcer Disease

(c) 2007, Michael A. Kahn, DDS/Lynn W. Solomon, DDS

Epithelial cells are continuously *renewed* every 4–5 days through a process of cell division, maturation, ... Eventually, they undergo apoptosis and are *shed* off into the intestinal lumen

II. Screening of antiulcer effect

- 1. Gastric lesions induced by NSAIDs (aspirin, indomethacin); ethanol
- **2.** Gastric lesions induced by stress.
- 3. Shay ulcer or pyloric ligation.
- 4. Isolated tissue (actions mediated via H2 receptors)
- a. stomach acid secretion
- b. isolated atria
- c. isolated rat uterus

Induction of gastric lesions

Chemically or pharmacologically -induced

NSAIDs (ASA,400 or Indomethacin 200 mg/kg, PO, EthOH, Reserpine, Cysteamine

Test drug is given 30 min before aspirin or necrotizing agent.

Stress-induced

- a. Cold restraint stress (CRS)
- b. Activity stress ulcers (chronic)

c. Shock-induced stress ulcers:

- **1. Electric**
- 2. Haemorrhagic
- 3. Septic (bile+bacteria IP, 2days)

Pyloric ligation or Shay method

Acid secretion + ulcerogenic effect

1. chemically- induced gastric ulcers (*Ethanol-induced gastric ulcer model*)

400 mg/kg aspirin, 200 mg/kg indomethacin or 1ml/200g absolute ethanol

Oral or gastric gavage

Ulcer due to ethanol is the result of reactive oxygen species (ROS) generation, microvascular injury and release of inflammatory mediators, which leads to increased vascular permeability, edema formation, and epithelial lifting resulting in necrotic lesions in the gastric mucosa.

2. Stress induced gastric ulcers

- a. Cold restraint stress (CRS).
- **b.** Activity stress ulcers (chronic)
- c. Shock-induced stress ulcers:
- 1. Electric
- 2. Haemorrhagic
- 3. Septic (bile+bacteria IP, 2days)

Mechanisms of stress ulcers

- 1. Reduced blood flow and ischemia is the main cause of stress-related ulcers.
- Reduced blood flow will result in diminished supply of O2 and nutrition and reduction of the removal of waste and toxins from tissue.
- 2. decreased production of mucus.
- 3. reduced production of bicarbonate.
- 4. reduced production of PGE2.

a. Cold Restraint Stress

(Immobilization)

* For induction of stress ulcers a 36 hr fasted rats were restrained in the supine position in a cold environment (6-9°C) for 3 hours.

* Following the restraint period animals were killed by an overdose of ether, the stomach removed, opened along the greater curvature, rinsed with warm distilled water and pinned flat on paraffin wax-filled petri dish and fixed in 10% formalin for 24 hours and examined for ulcer number and length.

b. Activity stress ulcers (chronic)

- -Rats are placed in a running wheel activity cage and fed ONLY 1 hr/day.
- -Ulcers develop in 4-12 days.
- -Rats run as much as 10 miles/day.
- -Ulcers penetrate into the <u>muscularis</u> <u>musosae</u> and thus differ from restraint ulcers which are superficial.
- The deep penetration of activitystress erosions is probably due to the chronic nature of these ulcers, i.e they develop slowly over period of several days.

Running wheel

b. Activity stress ulcers (chronic)

- -Rats are placed in a running wheel activity cage and fed ONLY 1 hr/day.
- -Ulcers develop in 4-12 days.
- -Rats run as much as 10 miles/day.<mark>≈16 km</mark>
- -Ulcers penetrate into the <u>muscularis</u> <u>musosae</u> and thus differ from restraint ulcers which are superficial.
- The deep penetration of activity-stress erosions is probably due to the chronic nature of these ulcers, i.e they develop slowly over period of several days.

c. Shock-induced stress ulcers:

1. Electric shock

rats , monkeys "executive monkey"

2. Haemorrhagic shock (dogs) haemorrhage until BP (40 mm Hg) for 4.5 hrs then reinfusion of shed blood. 3. Septic shock (dogs)

-Injection of a mixture of bile + bacteria (IP)

-Gastric erosions develop in 2 days

Assessment of gastric ulcers

Stomach is examined under a magnifying lens 3-10 X. The number and length of ulcers in (mm) for each animal is determined.

Curative ratio or % reduction or % inhibition

Curative ratio = <u>ulcer index (control)- ulcer index (treatment)</u>x 100

ulcer index of control

e.g. if ulcer index of control group = 8.5 mm.

And ulcers index in the test group = 2.5 mm.

8.5

::% inhibition = <u>8.5-2.5</u> x 100 = 70.59 %

Models of inducing gastric ulcers

III. Pyloric ligation "Shay's ulcer"

3. Shay Ulcer

- Rats are fasted for 24 hours before the experiment.
- Under anesthesia, abdomen is opened and stomach is ligated by tying off the pyloric portion.
- Replace stomach and close abdominal wall.
- After 18 hours animal is killed by cervical dislocation.

- Remove stomach and drain its contents into a test tube for determination of acid content and gastric volume.
- Stomach is then opened along the greater curvature for examination of ulcers.
- Test drug is given 2 days before and immediately after pyloric ligation.

Pylorus ligation causes accumulation of acid and pepsin, which leads to auto digestion of gastric mucosa and ulceration.

The hypersecretion was postulated to be due to stimulation of pressure receptors in the antral mucosa which initiated a vagovagal reflex.

4. Isolated tissue

Stimulation of H2 receptors in the stomach will stimulate acid and pepsin secretion. H2- blockers such as cimetidine will inhibit this effect.

Screening of H2- blockers involves the examination of their action on preparations whose response to histamine is mediated via H2 receptors such as:

- a. Stomach acid secretion: Shay method
- b. Isolated atria:
- c. Isolated rat uterus:

a. Stomach acid secretion Shay method

3. Shay Ulcer

- Rats are fasted for 24 hours before the experiment.
- Under anesthesia, abdomen is opened and stomach is ligated by tying off the pyloric portion.
- Replace stomach and close abdominal wall.
- After 18 hours animal is killed by cervical dislocation.

- Remove stomach and drain its contents into a test tube for determination of acid content and gastric volume.
- Stomach is then opened along the greater curvature for examination of ulcers.
- Test drug is given 2 days before and immediately after pyloric ligation.

b. Isolated rat atria

- Histamine causes acceleration of rat atrium. This effect is mediated by H2 receptors.
- Thus anithistamines (H2 blockers) will be screened by their ability to prevent this action when they are added to the organ bath before histamine.

The heart is cut out and the ventricular tissue cut away as far as possible. The auricles are placed in a dish containing Ringer-Locke solution at room temperature. A

thread is tied round the tip of each auricle (they should not be beating so this should not be difficult). The preparation is then transferred to the organ-bath and the beats should start once more.

Ringer-Locke solution : pure oxygen; 30" C

c. Isolated rat uterus

- Histamine causes relaxation of rat uterus and also inhibits Ach-induced contraction.
- H2-blockers prevent these actions of histamine.