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Review Article

In-Vivo Models Used for Pre-Clinical Evaluation of Anti-Ulcer Activity

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Abstract

Gastric ulcer disease has become a disease predominantly affecting the older population, with the peak incidence occurring between 55 and 65 years of age. It can occur in any part of gastrointestinal tract. Many medications are available for management of gastric ulcer. Prolonged use of these drugs may lead to serious adverse effects. Advanced in the discovery of more effective and safe anti-ulcer agent is due to the introduction of large number of newer experiment methods to evaluate their anti-ulcer activity in different types of gastric ulcers. Several in-vivo-models of gastric damage have been characterized and are primary tools to identify the anti-ulcer property of many new and existing drugs.

Keywords: Gastric Ulcer; Ulcer Score; Ulcer Index; Percentage Protection; Percentage Inhibition; Non-Steroidal Anti-Inflammatory Drugs

Introduction

Ulcers are lesions of the skin or mucous membrane characterized by the superficial inflamed dead tissue [1]. Peptic ulcer is the most predominant gastrointestinal disease [1,2]. Studies showed that gastric ulcer occurs at least 10% of the world population [4]. Peptic ulcer caused by a lack of balance between the gastric aggressive factors and gastric protective factors [3-5]. Aggressive factors include increased secretion of HCL and pepsin, inadequate dietary habits, free oxygen radicals, consumption of NSAIDs and alcohol, stress and infection of helicobacter pylori. Gastric protective factors include adequate gastric blood flow, secretion of prostaglandins, mucous, nitric oxide, bicarbonates and growth factors [6,7].

Drugs such as anticholinergic, histamine H_2 receptor antagonists, antacids and proton pump inhibitors are commonly used for treatment of peptic ulcer [8]. Prolong use of these drugs may lead to serious adverse effects like thrombocytopenia, nephrotoxicity, hepatotoxicity and impotence [8,9]. Due to unpleasant side effects of existing anti-ulcer drugs, there is need of more effective and safe treatment for ulcers.

There are several models used to evaluate anti-ulcer activity of existing as well as new drugs. This review mainly focus on various *in-vivo* models available for pre-clinical evaluation of anti-ulcer activity of drugs.

In-Vivo Models Used for Pre-Clinical Evaluation of Anti-Ulcer Activity

- Water-immersion stress or cold-restraint induced gastric ulcer model.
- Non Steroid Anti- Inflammatory Drugs (NSAIDs) induced gastric ulcer model.
- Ethanol induced gastric ulcer model.
- Acetic acid induced gastric ulcer model.
- Histamine induced gastric ulcer model.
- Reserpine induced gastric ulcer model.
- Serotonin induced gastric ulcer model.
- Pylorus ligated induced ulcer model.
- Diethyl dithiocarbonate (DDC) induced ulcer model.
- Methylene blue induced ulcer model.
- Ischemia-Reperfusion (I-R) induced gastric ulcer model.
- Cysteamine induced duodenal ulcer model.

Water-immersion stress or cold-restraint induced gastric ulcer model

Principle: In this model, gastric ulcers are induced by water-immersion stress or cold restraint stress in rats or mice. Stress induces ulcers by release of histamine which leads to an increased secretion of gastric acid, reduction in mucous production, reflux of pancreatic juice, and impairs gastric blood flow and increased gastro-intestinal motility [10-13].

Procedure:

Water-immersion stress induced ulcer model [14]**:**

- Animals are fasted for a period of 24-36 hours prior to the experiment.
- Animals treated with vehicle or test drug or reference drug.
- 30 minutes later, animals are placed individually in each compartment of a stress cage and immersed vertically up to xyphoid level in water bath and kept for 7 hours which result in induction of ulcers.
- 7 hours later, animals are sacrificed, stomach is dissected out and severity of ulcers is examined by calculating ulcer index.

Cold-restraint stress induced ulcer model [14,15]**:** Cold water immersion accelerates the development of ulcers in restraint animals. Wister rates are used for experiment.

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- Animals are fasted for 16 hours prior to the experiments.
- Test compound is administered orally.
- 1 hour later, animals are individually restraint in restraint cages vertically for 2 hours.
- Animals are immersed in water at 22ºc for 1 hour.
- Evans's blue, in dose of 30mg/kg is injected intravenously via the tail vein.
- Animals are sacrificed 10 minutes later.
- Stomach is removed and ligate at both ends.
- Stomach is filled with saline and kept overnight.
- On the next day, stomach is opened along the greater curvature, washed in warm water and examined for ulcer lesions.

NSAIDs induced gastric ulcer model

NSAIDs like aspirin, indomethacin and ibuprofen are the second most common cause of gastric ulcer [16].

Principle: NSAIDs cause ulcers by inhibiting prostaglandins synthesis by inhibiting cyclooxygenase enzyme in COX pathway. Prostaglandins play a protective role via stimulating the secretion of bicarbonates and mucous, maintaining blood flow and regulating mucous cell turn over and repair [17,18].

Procedure [16]**:**

- Animals are fasted for 24-36 hours.
- NSAID (aspirin or indomethacin) using appropriate vehicle (water or 1% carboxymethylcellulose) is orally administered.
- After 1 hour, animals are treated with test drug.
- 4 hours later, animals are sacrificed, stomach is removed and severity of ulcer is measured.
- Dose of NSAIDs used to induce ulcers:

Aspirin- 150mg/kg of body weight.

Indomethacin- 40-100mg/kg of body weight.

Ethanol induced gastric ulcer model

Principle: Ethanol causes ulcer lesions by exposing the gastric mucous to the hydrolytic and photolytic actions of HCl and pepsin [19,20].

Procedure [15,16]: Wister rats are used for experiment.

- Animals are fasted for 18 hours.
- Test drug is given to animals orally.
- 30 minutes later, 1ml of absolute ethanol is administered orally.
- 1 hour later, animals are sacrificed and their stomachs are dissected out.
- Stomach are opened along the greater curvature, washed with warm water and examined for ulcer severity.

Acetic acid induced gastric ulcer model

This method used for chronic peptic ulcers. This method is suitable to evaluate the effect of potential drugs and also to test the drug on the healing of chronic ulcers. Method can also use to screen ant secretory and ulcer protective effect of drugs [16,21].

Procedure [15,16]: albino rats are used for the experiment.

- Animals are fasted for 24-36 hours.
- Animals are anaesthetized
- A flexible plastic catheter with an outside diameter of 2mm is inserted up to 8cm in colon via anus, through which 2ml of diluted acid (4%) is introduced into colon.
- Animals are then kept into head down position for 2 minutes to prevent leakage of the acetic acid solution.
- After 24 hours, animals are sacrificed, stomach are removed and opened with greater curvature.
- Ulcer index is calculated to examine ulcer severity.

Histamine induced gastric ulcer model

Principle: Histamine released from mast cells and binds with receptors present on the surface of parietal cells which leads to activation of adenyl cyclase. This adenyl cylcase converts ATP into c-AMP. This conversion enhances secretion of HCL from parietal cells [22].

Procedure [15,16]: Male guniea pigs are used for the experiment.

- Animals are fasted for 36 hours.
- Histamine acid sulphate in dose of 50mg is injected intraperitonially.
- To prevent histamine toxicity, promethazine hydrochloride in dose of 5mg is injected intraperitonially 15 minutes before and 15 minutes after the histamine injection.
- Test drug is administered 30-45 minutes later of histamine injection.
- After 4 hours, animals are sacrificed, stomach removed and dissected
- Ulcer index is calculated to examine the severity of ulcers.

Reserpine induced gastric ulcer model

Principle: Reserpine acts on cholinergic system. Reserpine increases histamine secretion by causing degranulation of gastric mast cells [23].

Procedure [15,16]**:** Female Sprogue - Dawley rats are used for the experiment.

- Animals are fasted for 48 hours.
- Test drug is administered intraperitonially.
- Half an hour later, reserpine in dose of 15mg/kg is administered intraperitonially.
- 4 hours later, animals are sacrificed, stomach are removed and dissected.

- Ulcer index is calculated.

Serotonin induced gastric ulcer model

Principle [24]**:** Serotonin acts as vasoconstrictor which reduces gastric mucosal blood flow and leads to acute mucosal injury.

Procedure [16]**:**

- Animals are fasted for 24 hours.
- Serotonin creatin sulfate (0.5ml of 20-50mg/kg body weight) is administered subcutaneously.
- Animals are sacrificed after 6 hours, stomach is dissected out and examined for ulcer severity.

Pylorus ligated induced ulcer model

Principle [16]**:** The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach which leads to development of ulcers.

Procedure [15,16]**:** Wrister rats (150 to 180gm weight) are used for the experiment.

- Animals are fasted for 48 hours.
- Animals are anaesthetized and a 1 inch midline abdominal incision is given below the xyphoid process.
- The pylorus is carefully lifted out and ligated without damaging its blood supply.
- The stomach is now replaced and the abdominal wall closed with sutures.
- The test compound is administered either orally or subcutaneously.
- 10-19 hours later, animals are sacrificed and stomachs are dissected out.
- Contents of the stomach are drained into a graduated centrifuge tube and their acidity determined by titration with 0.1N NaOH.
- Stomach is opened along its greature curvature and ulcer index is calculated.

Diethyl Dithiocarbonate (DDC) induced ulcer model

Principle [25]**:** DDC induces ulcers through the mobilization of super-oxide and hydroxyl radicals. Super-oxide radicals and hydroxyl radicals plays a pathogenic role in development of ulcers.

Procedure [14,26]**:** This model is used to assess the anti-oxidative activity and cyto-protective activity of drug.

- Animals are fasted for 24 hours.
- Acute glandular lesions are induced by subcutaneous injection of 1ml of DDC in saline followed by oral dose of 1ml of 0.1N HCL.

Methylene Blue induced ulcer model

Principle [27,28]**:** Methylene blue is a synthetic drug. It is known to generate super-oxide radical ions by uncoupling of ATPase. In addition it also have anti-cholinergic activity. Drugs with anti-cholineregic activity and proton pump inhibitory activity can be assessed bu using this model.

Procedure [16]**:**

- Animals are fasted for 24 hours.
- Methylene blue is administered at a dose of 125mg/kg of body weight orally followed by the administration of test drug.
- Animals are sacrificed after 4 hours of methylene blue administration.
- Stomachs are dissected out and ulcer index is calculated.

Ischemia-Reperfusion (I-R) induced gastric ulcer model

Principle [29]**:** Reperfusion of gastro intestine following ishemia leads to formation of free radicals which results in development of erosion and ulceration in the gastric mucosa.

Procedure [30]**:**

- Animals are fasted for 24 hours.
- Animals are anesthetized
- Laparatomy is performed and esophageal and pyloric ends of the stomach are clamped using bull god clips.
- Celiac artery is then clamped at a point of 0.5cm distal from the branch to the aorta for 30min.
- GI is then reperfused for 20min.
- Animals are sacrificed, stomachs are dissected out and ulcer index is calculated.

Cytamine induced duodenal ulcer model

Principle [31,32]**:** Cytamine develops formation of duodenal ulcers by stimulating gastric acid secretion and inhibiting the secretion of alkaline mucous from brunner's gland.

Procedure [33]**:** There are two types of duodenal ulcers i.e. acute and chronic.

- Acute ulcers can be produced by administering single dose (400mg/kg of body weight) of cytamine HCL.
- Chronic ulcers can be produced by administering (400mg/kg of body weight) of cytamine HCL twice at an interval of 4 hours.
- Cut opened along the antimesenteric side and ulcer areas are measured.

Parameters to be Calculated

There are three parameters i.e., ulcer index, % protection ratio and % curative ratio, calculated by using method described by Tokagi and Okabe to evaluate anti-ulcer activity of drug in *in-vivo* models.

Steps:

- Give score based on ulcer severity.
- Calculate Ulcer Index (UI) based on ulcer score.
- % protection ratio and % curative ratio can be calculated by using Ulcer Index (UI).

Scoring of ulcers based on ulcer severity

Score	Ulcer severity
0	No lesions
1	mucosal oedema
2	1-5 small lesions (1-2 mm in size)
3	> 5 small or intermediate (3-4 mm in size) lesions
4	≥ 2 intermediate lesions or 1 gross (> 4 mm in size) lesion
5	Perforated lesions

Calculation of Ulcer Index (UI) based on ulcer score

By using ulcer score as described above, ulcer index can be calculated as following:

$$\text{Ulcer Index (UI)} = \frac{\text{Total ulcer score}}{\text{Number of animals ulcerated}}$$

Calculation of % protection ratio and % curative ratio by using Ulcer Index

% protection ration =

$$\frac{\text{UI of ulcerogen treated group}}{\text{UI of ulcerogen treated}} - \frac{\text{UI of drug pre treated group}}{\text{UI of ulcerogen treated}}$$

% curative ration =

$$\frac{\text{UI of ulcerogen treated group}}{\text{UI of ulcerogen treated}} - \frac{\text{UI of drug treated group}}{\text{UI of ulcerogen treated}}$$

Discussion

Ulcer is an important gastrointestinal disease that mainly caused by *H. Pylori* infection and high intake of NSAIDs. Pre-clinical evaluation of new or existing anti-ulcer drug can be done by using appropriate *in-vivo* models. Several models are developed to evaluate anti-ulcer activity of natural as well as synthetic drugs. *In-vivo* models may also be used to assess any toxic effects of test drug.

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