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Review Article

A Review on Current and Evolving Techniques in Antiulcer Drug Screening

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ABSTRACT

Peptic ulcer, defined as a lesion in the gastric or duodenal mucosa caused by an imbalance between aggressive and protective factors, remains a global health concern. The continued prevalence of peptic ulcer disease highlights the urgent need for improved therapeutic strategies and reliable preclinical screening models. This article reviews innovative methodologies currently employed in the evaluation of antiulcer drug candidates. Among these, the zebrafish (Danio rerio) model offers significant advantages due to its genetic similarity to humans, ease of handling, and potential for high-throughput screening. Various chemically induced ulcer models, including those utilizing methylene blue, serotonin and cystamine, allow for the investigation of distinct pathological mechanisms such as oxidative damage, mucosal inflammation, and neurochemical imbalance. The ischemia-reperfusion injury model serves as a valuable tool for assessing the gastroprotective efficacy of compounds under conditions mimicking vascular impairment. Additionally, artificial gastric juice models provide a controlled in vitro environment for evaluating drug stability and mucosal interaction under simulated gastric conditions. All of these innovative methods work together to improve the accuracy and applicability of antiulcer medication screening and aid in the creation of more potent medicinal substances.

INTRODUCTION

A peptic ulcer is an open wound or sore that forms in the lining of the stomach or the upper section of the small intestine. It develops when the digestive acids break through the protective lining, often due to factors like infection with Helicobacter pylori bacteria or prolonged use of nonsteroidal anti-inflammatory drug.[1]

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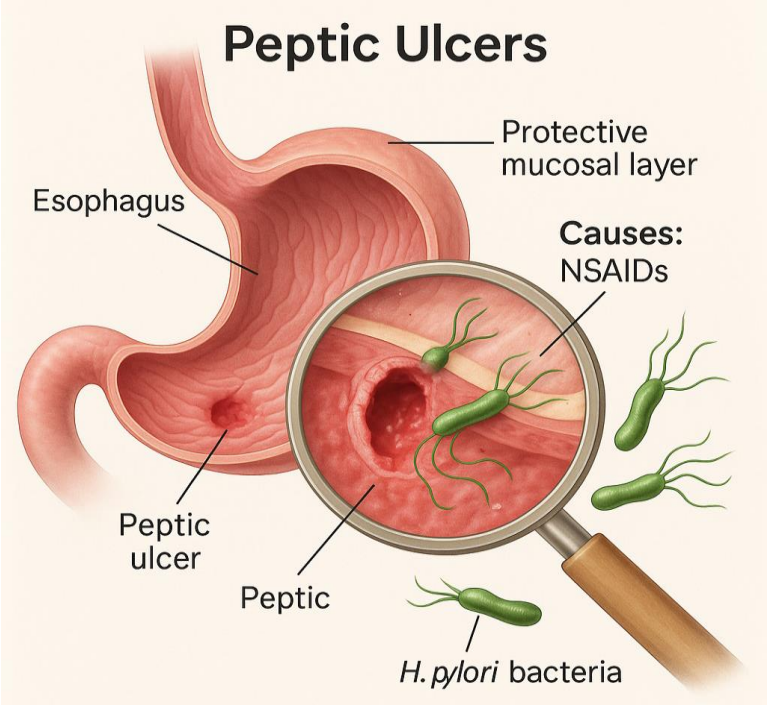

Despite available treatments, the high prevalence and recurrence of ulcers remain significant health concerns, driving the need for new and improved therapeutic options. Antiulcer drugs include several classes that act through different mechanisms to manage ulcers. Proton pump inhibitors such as omeprazole and lansoprazole, and H2-receptor antagonists like ranitidine and famotidine reduce gastric acid secretion. Antacids containing aluminium hydroxide or magnesium hydroxide neutralize stomach acid, while cytoprotective agents such as sucralfate and misoprostol strengthen the mucosal barrier. Additionally, antioxidants and anti-inflammatory compounds are used to reduce oxidative stress and inflammation that contribute to ulcer formation.[2,3] To discover and develop effective antiulcer agents, innovative screening methods are essential. Emerging experimental models such as zebrafish provide a versatile platform due to their genetic similarity to humans and suitability for rapid screening. Chemical induction methods mimic specific pathological pathways leading to ulceration, such as oxidative damage and neurotransmitter imbalance. Models simulating ischemia-reperfusion injury help evaluate drug efficacy under conditions of vascular stress, while artificial gastric juice systems offer controlled environments to assess drug stability and mucosal protection. Together, these approaches improve the predictive accuracy and translational relevance of preclinical antiulcer drug screening, supporting the advancement of safer and more effective therapies.

METHODS

Water Immersion Induced Ulcers

This method involves exposing rats or mice to either cold restraint stress or water immersion stress, which results in stomach ulcers. Histamine, which is released in response to stress, induces pancreatic juice reflux, decreases mucus production, increases gastric acid secretion, hinders stomach blood flow, and increases gastrointestinal motility. In general, stress exposure first reduces acid secretion; however, subsequent water immersion during the prestress phase causes an increase in acid secretion in

confined animals. In order to find medications that may be effective in treating gastropathy and stress-related stomach ulcers, this model is crucial.[4,13]

Procedure

Wistar rats weighing 150–200 g, of either sex, are used in the experiment. The rats are divided into groups of ten. Before the test begins, the rats are given a 24-hour fast. Following oral administration of the test medication or vehicle, the rats are rendered immobile in the stress cage and submerged vertically for 16 hours at 22°C in a water bath. Once the rats are taken out of the cage, we give them an intravenous injection of 30 mg/kg of Evan's blue into the tail vein. They are offered as sacrifices ten minutes later. After being removed, the stomach is tied off at both ends. It contains formol saline and is stored for the night. The following day, the stomachs are opened along their greatest curvature, cleaned with warm water, and examined under a three-fold magnifying glass. Each animal's total lesion score is determined by adding the lengths of the longest lesion diameters, with the mean count for each group being determined.[5]

Ischemia Reperfusion Model

Ischemia-reperfusion (I/R) modeling is a proven technique for antiulcer drug screening. Oxidative stress is triggered when the blood supply returns to normal in the gastrointestinal system after a time of limited flow. This can lead to erosions and ulcers in the gastric lining.[6]

Procedure

Male Wistar rats weighing 200–250g were fasted for 24 hours with free access to water prior to anesthesia with urethane. Following midline laparotomy, the stomach was exposed and instilled with 0.15 M HCl at a dose of 1 ml/100 g body weight. Ischemia was induced by occluding the left gastric artery using a vascular clamp for 5 minutes, followed by 30 minutes of reperfusion after clamp removal. Test compounds or standard treatments were administered immediately before ischemia induction in groups of five animals. At the conclusion of reperfusion, animals were sacrificed, and stomachs were fixed in 10% buffered formalin for subsequent analysis. Macroscopic mucosal injury was quantified via computer-assisted planimetry on photographic images and expressed as a percentage of total glandular mucosa. For histological evaluation, sections from the corpus region were stained with hematoxylin and eosin and scored based on epithelial damage, mucosal disruption, hemorrhage, and necrosis to generate a cumulative histological injury index (maximum score of 10). [7,13]

Zebra Fish Model

Zebrafish (Danio rerio) have emerged as a valuable and cost-effective model for gastrointestinal research, offering several advantages over traditional mammalian systems. Zebrafish embryos, due to their optical clarity, swift maturation, and relevant biological responses to gastric damage, serve as an effective system for examining the processes involved in ulcer initiation and tissue regeneration. Additionally, their rapid growth and conserved responses to ulcerogenic stimuli make them an effective system for studying gastrointestinal disorders and for screening potential anti-ulcer agents. [8,9]

Procedure

Three tanks containing four zebra fish were designated as the control, induction, and treatment groups. A solution of 2% w/v Dextran Sulfate sodium (DSS) is used to induce ulcers. It is mixed with the water in the treatment and induction

tanks. The concentration of DSS utilized was determined by other relevant studies. The control group was kept in untreated water to make sure that the effects were Ulcer formation was estimated, and marked ulcerative lesions were evident within 7 days of DSS treatment. To create 14 consistent feed balls, 840 µL of extract and 1 g of powdered feed were combined. For seven days, zebrafish were given two balls every day while the dosage was closely monitored.[9]

Analysis: The intestines were moved to centrifuge tubes containing lysis buffer after the fish were slaughtered on the twenty-first day. After homogenizing the mixture and centrifuging it, the suspension was obtained for biochemical tests like the myeloperoxidase (MPO) assay, nitric oxide (NO) estimation.[9] Tissue sections were deparaffinized in xylene, rehydrated through a graded alcohol series, and stained with Mayer's hematoxylin and eosin. After dehydration and clearing, slides were mounted with DPX and examined under a light microscope for histological analysis.[9]

The Neutralization Duration Capacity of Prepared Artificial Stomach Acid

The Vatiers artificial stomach device is an experimental setup used to measure the time required to neutralize gastric acid. It is composed of three compartments: a reservoir tank for holding fluids (S-1), a drainage area to collect stomach contents (S-2), and a secretory chamber where digestive fluids flow (S-3). A roller pump simulates the stomach's movements, and a pH meter continuously records changes during the process.[4]

Procedure

The reference medications are placed in a beaker and heated to 37 °C in a water bath while a magnetic mixer runs continuously at 30 revolutions per minute to simulate stomach activity. The test drug is newly extracted from the plant for titration. Laboratory-prepared stomach juice is used to titrate each plant extract and reference medication separately until the endpoint pH value reaches 3. Ultimately, after titration, the neutralized capacity, total hydrogen ion consumption, and total volume of laboratory stomach juice consumed are measured.[4]

Methylene Blue Induced Ulcer

The synthetic drug MB is known to produce superoxide radical ions and uncouple ATPases. To investigate the mechanisms of action of antiulcer medicines, MB is employed as an ulcerogenic agent. This pharmacological method is used to screen for different antiulcer drugs that alter the H+/K+ ATPase system. Animals given MB experience duodenal and stomach lesions. Moreover, the substance suppresses the action of nitric oxide synthase, which lowers the supply of nitric oxide in the body. Furthermore, MB has a preference for muscarinic or cholinergic receptors and is said to suppress cholinesterase activity suggests that antiulcer medications with proton-pump inhibitory action and anticholinergic effects may be evaluated using this animal. MB induces oxidative stress, which in turn leads to erosion and ulceration of the stomach mucosa by reducing the blood flow to the mucosa. [10,13]

Procedure

Animals are fasted for 24 hours prior to the experiment and given unlimited water in order to induce ulcers with MB. Following the oral administration of MB at a dose of 125 mg/kg body weight, the medication or test substances are delivered. Following four hours of MB administration, the animals are sacrificed. The ulcer index is calculated by dissecting the

experimental animals' stomachs and cutting them open by the greater curvature. [7]

Cystamine Induced Ulcer

Duodenal ulcers in rodents that are experimentally created by administering cystamine hydrochloride are known as cystamine-induced ulcers. This model is frequently used in pharmacological research to assess the effectiveness of antiulcer medications and investigate the pathophysiology of peptic ulcer disease. [7]

Procedure

For the technique, Wistar or Sprague Dawley rats weighing around 200 g are used. On the first day of the experiment, give the fed rats 280 mg/kg of cysteamine HCL orally three times a day. Give the medications to the treated animals 30 minutes prior to the first dose and again 24 hours later on the second day. After receiving the initial dose of cysteamine HCL for 48 hours, the rats are killed. The duodenal ulcers often rupture the liver and form on the anterior wall of the duodenum, 2–4 mm from the pylorus. A kissing ulcer is a small ulcer that develops on the duodenum's posterior wall and always spreads to the pancreas. The duodenal ulcers' severity is assessed. The intensity of the duodenal ulcers is evaluated. [11,7,13]

0 – No ulcer
1 – Superficial mucosal erosion
2 – Deep ulcer usually with transmural
3 – Perforated or penetrated ulcer

Serotonin Induced Ulcer

The monoamine serotonin, also referred to as 5-hydroxytryptamine (5-HT), is made from the necessary amino acid tryptophan. It is produced by chromaffin cells in the digestive tract as well as serotonergic neurons in the central nervous system, where it serves as a neurotransmitter. Because serotonin causes vasoconstriction, it affects blood vessels by decreasing blood flow to the stomach mucosa. Lesions in the mucosa may result from this decrease in blood supply. After a 36-hour interval of no meals, serotonin is usually given via stomach intubation. [7,13]

Procedure

Rats are weighed and fasted for 24 to 36 hours before the experiment with unlimited water in this method. Two hours before the studies start, the fasting animals are not given any water. Serotonin creatinine sulfate (0.5 mL of 50 mg/kg subcutaneous injection) is administered once, and glandular lesions are formed as a result. With the use of an orogastric cannula, serotonin is delivered through intra-gastric intubation. Six hours later, the animals are dislocated cervically to be sacrificed. The stomach is removed in order to conduct more ulcer index research. [12,13]

CONCLUSION

Advancing the treatment of peptic ulcer disease relies heavily on the development of reliable and innovative preclinical screening models. This review highlights several modern approaches that enhance the understanding of ulcer formation and support the identification of effective therapeutic agents. Animal-based models such as water immersion stress, ischemia-reperfusion injury, and chemically induced ulcers using agents like methylene blue, cystamine, and serotonin help simulate different pathological pathways. The zebrafish model, with its genetic similarity to humans and suitability for rapid screening, offers a promising alternative to traditional methods. In vitro systems like artificial gastric juice setups provide a controlled environment for evaluating the acid-neutralizing capacity and mucosal protection of test compounds. These diverse strategies not only improve the predictive value of antiulcer drug screening but also pave the way for

the development of safer and more targeted ulcer therapies.

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