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Review article

PRECLINICAL RESEARCH TECHNIQUES FOR INVESTIGATING THERAPEUTIC LEADS AGAINST GASTROINTESTINAL ULCER

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Abstract

Background: *An ulcer is an ongoing health ailment affecting all age groups wherein the linings of the oesophagus, stomach, and intestine are mainly affected. Many discoveries were made for ulcer therapy but research findings on a disease soon become irrelevant due to ever-changing pathology. Therefore, there is a need for newer drugs for pharmacological interventions. However, discovering or developing a new drug requires ethically and scientifically acceptable pre-clinical tools for assessing therapeutic activity.* ***Objective:*** *The review aims to compile and highlight the pre-clinical techniques/models used for assessing the efficacy of potential anti-ulcer agents.* ***Materials and Methods:*** *A comprehensive theoretical survey was done from October 2019 up to April 2020. Keywords such as, 'ulcer', 'in-vitro evaluations', and 'in vivo evaluations' were used either alone or in combination. Academic search engines like PubMed, Science Direct, Google Scholar, Web of Science, and Scopus were utilized.* ***Results and Discussion:*** *The exhaustive literature study provides in vitro and in-vivo techniques for evaluating potential anti-ulcer agents. Successfully employed ulcer inducers, test drugs, biological preparations or animal models for examining the pharmacological activity of anti-ulcer agents are highlighted.* ***Conclusion:*** *Researchers working to discover and develop newer/safer anti-ulcer agents may utilize this literature for retrieving appropriate in vitro or in-vivo pre-clinical techniques for investigating potential anti-ulcer agents.*

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Keywords: Drug discovery; Experimental pharmacology; Gastric anomaly; *in-vitro*; *in-vivo*

Introduction

An ulcer is mainly caused due to an imbalance between the acid and pepsin and/or due to the weakness of the mucosal barrier [1].

Identification and diagnosis are always of primary importance for any disease. For this reason, researchers or epidemiologists may search for miser clue. Clinical investigations seem to improve with questionnaires designed to identify successfully used methods [2]. However, the demography of results related to ulcers is not stable and changes from time-to-time mainly due to age differences, sex, site of the gastric lesion, blood groups, depression, and social engagement [2–4].

Even though many discoveries were made, these discoveries do have some demerits in them. With time, research findings of an ailment can become irrelevant due to ever-changing pathology. Clinical manifestations of a disease may also differ from a different population [2].

Factors like urbanization, climatic condition, topography, occupational behaviors, psychogenic factors, family genetics, smoking of cigarettes, alcohol consumption, and regular coffee intake contribute to peptic ulcer [2,5]. Peptic ulcer remains to be the main cause of morbidity and mortality worldwide [6].

Different drugs and clinical methods available forprophylaxis, mitigation, and treatment will be employed as long as the benefit outweighs the adverse effects. Polypharmacy is also often practiced due to the unavailability of a single medication efficacious enough to ameliorate gastrointestinal (GI) ulcers. Discovering newer or developing safer drugs might help in dodging the adverse effects of existing pharmacotherapy for GI ulcers. Therefore, finding and establishing the efficacy of new leads with enhanced safety and therapeutic potential requires ethically approved *in-vitro* and *in-vivo* techniques.

Materials and Methods

Keywords like ‘Ulcer’ alone and/or in combination with *In Vitro* evaluations and/or *In Vivo* evaluations were entered in academic search engines *viz.* PubMed, Scopus, Google Scholar, Web of Science, and Science Direct. A comprehensive theoretical analysis was carried out from October 2019 until April 2020.

A review on ulcers was also done to help better understand the main topic of the current communications.

Definition of ulcer

An ulcer is defined as a disease that occurs due to the breakdown of the epithelial mucosal barrier of the stomach when exposed to an excess of hydrochloric acid and/or pepsin [7, 8].The imbalance between mucosal defence and aggressive factors also contribute to abnormalities of the gastric mucosa [8].

Types of ulcer

Ulcers may manifest as a peptic ulcer, which includes duodenal ulcer and gastric ulcer and *Helicobacter pylori (H. pylori)* induced ulcer [9–11]. The occurrence of ulcer is uncommon in children in comparison to adults. Though the most common cause of ulcer is an infection by *H. pylori* and irrational use of NSAIDs, ulcers can be induced by other factors as well [7,8]. Therefore, ulcers may be classified into primary and secondary ulcers according to the underlying aetiology.

Primary ulcers are chronic, rare in children less than 10 years of age, include *H. pylori* induced ulcer and *Helicobacter* negative gastritis. Hyper-secretory abnormalities like Zollinger-Ellison Syndrome (ZES), G-cell hyperplasia, systemic mastocytosis, hyperparathyroidism, and cystic fibrosis is also included [8].

Secondary peptic ulcers are acute, occurs at any age with higher rates of mortality in children. The systemic underlying the disease is a great contributor to secondary peptic ulcers like *Helicobacter heilmannii* gastritis, gastric tuberculosis, viral gastritis, cytomegalovirus infection, parasitic gastritis, fungal gastritis, drug-induced gastropathy, stress gastritis and/or ulceration, bile gastropathy, radiation gastropathy, lymphocytic gastritis, autoimmune atrophic gastritis, eosinophilic gastritis, vascular gastropathy, and collagenous gastritis [8].

Associated factors of ulcer

Factors like *H. pylori* infection and irrational use of NSAIDs for a long-term speed up the onset and severity of ulcer [12–14]. Dysbiosis is an imbalanced gastrointestinal micro bio-data and may also induce ulcers. *H. pylori* is the bacterium having the highest rate of abundance in patients diagnosed with gastric ulcer [15–17]. *H. pylori* may infect the proximal (cardia) and distal (non-cardia) part and/or site of the body [18]. It may also cause mucosal-associated lymphoid tissue lymphoma [19,20].

However, *H. pylori*-induced ulcer in developed countries are less but NSAIDs induced ulcers are common [12]. The aetiological agents and/or factors involved in the development of ulcer are represented in Table 1.

Table 1: Factors associated with the development and/or progression of peptic ulcer disease

Sl. No.	Factor	Causative agent
1	Infection	*H. pylori* infection [7,12]
		Cytomegalovirus [7]
		Herpes simplex virus [7]
		Helicobacter heilmannii[8]
2	Drugs	Non-steroidal anti-inflammatory agent (NSAIDs) [7,12]
		Acetylsalicylic acid (Aspirin) [7,12]
		Corticosteroids [7]
		Recreational drugs [12]
3	Diseases	Gastric adenocarcinoma [12]
		Local drug irritation [12]
		Systemic mastocytosis [25]
		Multiple endocrine neoplasia type-I [25]
		Obesity [24]
		Diabetes [24]
4	Hyper-secretory state	Zollinger-Ellison Syndrome [7,21]
		Hyperparathyroidism [25]
		Cystic fibrosis [8]
5	Post surgical	Anastomotic marginal following surgery [7]
6	Autoimmune diseases	Crohn's disease [7,12]
7	Stress-induced	Psychologic stress [21]

		Trauma [7]
		Sepsis [7]
		Multiple organ failure [7]
8	Lifestyle	Alcohol intake [7,21]
		Smoking [7,12,21]
9	Other causes	After radiotherapy [12]
		Hepatic artery chemotherapy [25]

Clinical manifestations of ulcer

Two-thirds of patients diagnosed with peptic ulcer are asymptomatic [6]. Majority of ulcer patients have abdominal pain and/or episodic epigastric pain, postprandial pain, and nocturnal pain [21–23]. Common symptoms of ulcer in 80-90% of patients include episodic gnawing, dull, burning epigastric pain [24].

Symptoms and/or features of the ulcer may also include indigestion, belching, vomiting associated with gastric or pyloric stenosis, nausea, loss of appetite, intolerance of fatty foods, anaemia caused by GI blood loss, anorexia, and progressive weight loss [12,21,24,25].

Inflammatory ulcers produce symptoms such as weight loss, cachexia, and chronic abdominal pain. Among various symptoms, abdominal pain is prevalent in younger patients. Older patients (<80 years) with ulcer commonly shows symptoms like epigastric pain (74%), nausea (23%), and vomiting (20%). However, the symptoms of ulcer remain mild in pregnant patients [24].

Complications associated with gastrointestinal ulcer

NSAIDs induced ulcers may result in serious complications while *H. pylori-induced* ulcer heal and relapse spontaneously. Acute bleeding ulcers may cause haematemesis while chronic bleeding can result in anaemia [12]. Chronic infection of *H. pylori* can lead to chronic atrophic gastritis (AG), gastric intestinal metaplasia and ischemic stroke [19, 26–28]. Besides, an ulcer may cause bleeding, perforation, penetration, gastric outlet obstruction, ZES, hypotensive shock, metabolic acidosis, acute renal failure, hypo-albuminemia, acute upper gastrointestinal haemorrhage, tachycardia, abdominal rigidity, abdominal distension, tenderness to palpation, gastritis, and tissue damage [6,29,30]. Another possible complication accompanying ulcer is gastric cancer which ranks fifth as leading cancer type and is possibly linked with the death of many patients [15].

Prevention of ulcer

Vaccination against *H. pylori* may become a potentially useful strategy in preventing ulcers in the future, though further research is needed. Rational prescribing (RP) of drugs can reduce the incidence of drug-induced ulcers. RP of drugs is advantageous in patients with increased risk of ulcers like the elderly and/or those with the previous history of the ulcer [12].

Proton pump inhibitors (PPIs) are recommended for symptomatic, elderly patients in need of NSAIDs and/or aspirin. For patients with a history of bleeding, a combined prescription of coxibs and PPIs is regarded as the best approach. However, the best strategy for preventing ulcers is still debated [21].

Current therapy of ulcer

Treatment and diagnostic tools that are available and currently in use include the class of drugs like PPIs [6,31]. Antacids, anticholinergics, potassium-competitive acid blocker, histamine-2-receptor antagonists (H_2RAs), antibiotics like clarithromycin, penicillin, amoxicillin, tetracyclines and metronidazole may be used alone or in combination with other drugs [6,31]. Endoscopic interventions are preferred for patients with cases of upper GI tract bleeding [6,32]. Although it has faced many challenges, immunization using vaccines against *H. pylori* has been tested in mice and was found to be effective in inducing some protective immunity [33]. In the modern era, nanoencapsulation is applied in peptic ulcer treatment with constituents mainly derived from natural sources [14].

Pre-clinical models for the evaluation of anti-ulcer agents

In any drug development, a pre-clinical study is a stage of research that is carried out before clinical trials. The main aim and importance of pre-clinical evaluation and/or testing is to collect data in support of the safety of the the agent under investigation.

***In-vitro* techniques**

The use of preclinical techniques for *in vitro* evaluation of anti-ulcer agent is limited (Table 2). Literature records available reveal the use of indomethacin as the primary inducer for ulcer. For *in vitro* technique, test drugs are evaluated using various biological preparations.

Table 2: *In vitro* evaluation techniques for gastric ulcer

Sl. No.	Inducer/ Techniques	Test drug(s)	Biological preparation(s)

1	Indomethacin	Sucralfate formulations, aluminium hydroxide, potassium sucrose octasulfateheptahydrate and 5-aminosalicylic acid	Cultures of gastric cells from rats [34]
2		Sucralfate, rebamipide, cimetidine	Gastric mucosal cells of rats [35]
3	Indomethacin	Meloxicam, Indomethacin, Ketoprofen, irsogladine, rebamipide	Human iPS cells [36]
4	Mucin 2 assay	Rebamipide	Gastric mucosal cells of rats [37]
5		Leminoprazole	Rabbit gastric mucosal cells [38]
6	Indomethacin	Rebamipide on paracellular permeability of rat gastric epithelial cells	Rat gastric epithelial cells [39]
	Indomethacin		
	Indomethacin		

***In vivo* techniques**

In vivo techniques for evaluation of anti-ulcer agents are widely used (Table 3). From the literature survey, a total of 28 ulcer inducers havebeen reported. A series of test drugs had been subjected for scientific evaluation in different species of rodents like rats, mice and guinea pigs.

Table 3: *In vivo* evaluation techniques for gastric ulcer

Sl. No.	**Inducer**	**Test drug(s)**	**Animal(s)**

1	Histamine	Ferulic acid	Rats [40]
		Garcinia indica	Rats [41]
		Tagathen	Guinea pig [42]
		Somatostatin	Rats [43]
		Brompheniramine, AHR-224, tripelenamineHCl, cyproheptadineHCl and Promethazine HCl	Guinea pig [44]
		Dimaprit	Guinea pig [45]
		Benincasa hispida	Rats [46]
2	Indometha cin	Polyherbal formulation	Albino rats [47]
		Avicenna mariana	Albino rats [48]
		Bauhinia purpurea leaf	Sprague-Dawleyrats [49]
		Cyperus alternifolius	Rats [50]
		Garlic	Rats [51]
		Rebamipide	Rats [52]
		Spondias mombin and *Ficus exasperate*	Wistar rats [53]
		Telmisartan	Rats [54]
		Annona muricata	Mice [55]
		Safranal	Rats [56]
		Nicorandil	Rats [57]
		Leucas aspera	Rats [58]
		Benincasa hispida	Rats [46]
		Asparagus pubescens	Rats [59]
		Melatonin	Rats [60]
3	Ethanol	Polyherbal formulations	Albino rats [47]
		Bauhinia purpurea	Sprague-Dawley rats [49]

Tetrahydrocoptisine	Mice [61]
Asparagus pubescens	Rats [59]
Methylene blue	Rats [62]
Garcinia indica	Rats [41]
Hericium erinaceus	Rats [63]
Jasminum grandiflorum	Rats [64]
Scutia buxifolia	Rats [65]
Vanillin	Rats [66]
Bovine milk	Mice [67]
Nobiletin	Mice [68]
Annona muricata	Mice [55]
Lactobacillus reuteri	Mice [69]
β-glucan	Rats [70]
Aucubin	Mice [71]
Dioscorea batatas	Mice [72]
Calein D	Rats [73]
Gallic acid	Rats [74]
Nicorandil	Rats [57]
Cimetidine	Rats [75]
Loranthus acacia	Rats [76]
Centella asiatica	Rats [77]
Chelerythrine	Mice [78]
Pantoprazole	Rats [79]
Fisetin	Rats [80]
Wogonin (Flavonoid)	Rats [81]
Zinger officinale	Rats [82]
Patchoulene epoxide	Rats [83]
Dentatin	Rats [84]

		Pyranocycloartobiloxanthone A	Rats [85]
		Samanea saman	Albino rats [86]
4	Ischemia-reperfusion	Polyherbal formulation	Albino rats [47]
		Role of Leukocytes	Rats [87]
		Zinc-carnosine chelate	Rats [78,88]
5	Aspirin	*Garcinia indica*	Rats [41]
		Jasminum grandiflorum	Rats [64]
		Polyalthia longifolia	Rats [89]
6	Pylorus ligation	*Hericium erinaceus*	Rats [63]
		Jasminum grandiflorum	Rats [64]
		Quinoline-chalcone	Rats [90]
		Montelukast	Rats [91]
		Brompheniramine, AHR-224, tripelenamineHCl, cyproheptadineHCl and Promethazine HCl	Rats [44]
		Fisetin	Rats [80]
		Free radicals and antioxidant	Rats [92]
		Polyalthia longifolia	Rats [89]
		Pantoprazole	Rats [79]
7	Naproxen	*Madhuca indica*	Rats[93]
8	Radiation	Rebamipide	Mice [94]
9	*Helicobacter pylori*	Turmeric	Rats [95]
		Pioglitazone	Rats [96]
		Ginger rhizome *(Zinger officinale)*	Rats [82]
		Omeprazole	Rats [97]
10	Acetic acid	Hesperidin	Rats [98]
11	Acetylsalicylic acid	Bee venom	Rats [99]

12	Cysteamine	*Leucas aspera*	Rats[58,100,101]
13	Restraint	Food deprivation	Rats [102]
		Brompheniramine, AHR-224, tripelenamineHCl, cyproheptadineHCl and Promethazine HCl	Guinea pig [44]
		Morphine	Rats [103]
		Benincasa hispida	Rats [46]
		Body temperature	Rats [104]
		Mild whole body heating	Rats [105]
14	Hydrochloric acid (HCl)	Bovine milk	Mice [67]
		Polyalthia longifolia	Rats [89]
15	Ferrous ion	New gastric ulcer model(lipid peroxide)	Rats [106]
16	Methylene blue	Methylene blue	Rats [107]
17	Gastric distension	HCl	Rats [108]
18	Water immersion stress	Montelukast	Rats [91]
		Cyclodextrin	Rats [109]
		Nitrous oxide synthase	Rats [110]
		Polyalthia longifolia	Rats [89]
		Mild whole body heating	Rats [105]
		Enhancing nitric oxide synthase	Rats [110]
		Prostaglandin level	Rats [111]
		Kaempferia parviflora	Rats [112]
		Gynostemma pentaphyllum	Rats [113]
		Body temperature	Rats [114]
		Samanea saman	Albino rats [86]

19	Reserpine	Dragon-pearl tea crude polyphenol extract	Mice [115]
		Insect tea	Mice [116]
		Larimichthys crocea	Mice [117]
		Thenalidine	Rats [118]
		Degranulation of mucosal mast cells	Rats [119]
		Age, sex and cold exposure	Rats [120]
		Brompheniramine, AHR-224, tripelenamineHCl, cyproheptadineHCl and Promethazine HCl	Rats [44]
		Mild whole body heating	Rats [105]
20	Serotonin	Thenalidine	Mice [118]
		Prastagladin E_1	Rats [121]
		Somatostatin	Rats [43]
		Leucas aspera	Rats [58]
		Benincasa hispida	Rats [46]
21	Diethyldithiocarbamate	Active oxygen species	Rats [122]
		Rebamipide	Rats [123]
22	Stress induced	Zinc deficiency	Rats [124]
		Cimetidine	Rats [75]
		Leucas aspera	Rats [58]
		Pantoprazole	Rats [79]
		Cyclodextrin complexation	Rats [125]
		Chlorealla vulgaris	Rats [126]
		Atropine and Antacids	Rats [127]
		Zinger officinale	Rats [82]
		Reactive oxygen species	Rats [128]
		Omeprazole	Rats [97]

23	Swimming induced	*Leucas aspera*	Mice [58]
		Benincasa hispida	Rats [46]
		Decalepis hamiltonii	Rats [129]
		Guduchi ghrita	Rats [130]
24	NSAIDs	5-lipoxygenase inhibitors and leukotrienes antagonist	Mice [131]
		Cyclodextrin	Rats [109]
		Gallic acid	Rats [132]
		Misoprostol	Humans [133]
		Cyclodextrin complexation	Rats [109]
		Omeprazole	Rats [97]
25	Cold restraint	Flavonoid (Wogonin)	Rats [81]
		Cimetidine	Rats [75]
26	Piroxicam	Melatonin	Rats [134]
		Sulphydryl and neutrophil infiltration	Rats [135]
		Aqueous tulsi leaf	Rats [136]
		Cyclodextrin complexation	Rats [110]
27	Restraint-stress	Gastric motility	Rats [137]
28	Adjuvant Carrageenan	Hypolaetin-8-glucoside	Rats [138]

Discussion

The *in vitro* techniques seem to be rarely used for investigation of anti-ulcer agents (refer to Table 2). *In vitro* evaluation of anti-ulcer drugs is rarely used as satisfactory results cannot be obtained using test-tube experiments [139]. Moreover, it is difficult to stimulate the consequences of long term exposure [140].

In vivo techniques are commonly used, as the diseased condition in humans can be mimicked using different animal models [141]. Owing to their anatomical, physiological, and genetic similarities to humans, rodents like mice and rats are

preferred among the various animal models. Maintenance and handling of rodents are easy due to their convenient smaller size [142]. Interestingly, mice share more than 98% DNA similarities with humans [143]. Administration of ethanol easily induce necrosis of the gastric cells, inflammation, reduces the secretion of bicarbonate, gastric mucus, and nitric oxide, consequently leading to ulceration. Hence, ethanol is most commonly used for the induction of ulcer in animal models [144,145].

Conclusion

Investigation for a potential anti-ulcer agent requires suitable *in vitro* or *in vivo* techniques. The current textual analyses systematically provide a compilation of techniques that are used in evaluating anti-ulcer agents. With crucial information made easily accessible like ulcer inducer, test drug and biological preparation or animal model employed, investigators of potential anti-ulcer agents may use this literature for reference to aid their research works. Though there was a possibility for a detailed description of each model used, the review is limited to highlight important and useful knowledge only.

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