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An Overview of the Current Methodologies Used for Evaluation of Gastric and Duodenal Anti-ulcer Agents

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Abstract: Background: Discoveries in the past two decades have continued to improve our understanding of the pathophysiology of peptic ulcer disease and animal models have played a significant role to define the basic mechanisms of gastric mucosal defense and repair. In the early 20th century, peptic ulcer disease was related to excessive acid secretion resulting from stressful lifestyle. The importance of the interaction of acid and pepsin in the formation of peptic ulcer disease remained unclear until the second half of the 20th century. With the introduction of histamine type 2 receptor antagonists (H_2 receptor antagonists) in the 1970s, progress was made in reducing acid secretion and providing relief of symptoms. With further advancements in medical research, it is now well recognized that use of nonsteroidal anti-inflammatory drugs (NSAIDs) contribute to the development of peptic ulcer disease. Moreover, the identification of the role of a bacterium, *Helicobacter pylori* (*H. pylori*) in the pathogenesis of acid-peptic diseases, its elimination with antimicrobial drugs could effectively cure the disease stimulated new approaches to prevention and therapy. These advances in the discovery of novel and more effective anti-ulcer therapeutics is due to the introduction of a larger number of newer experimental methods to evaluate their anti-ulcer activity in different types of gastro-duodenal ulcers and simultaneously to study their mechanism of action. Several *in vivo* models of gastric damage have been well characterized and are the primary tools used by gastrointestinal physiologists, pharmacologists and pathologists to study new mechanisms of pathogenesis and new pharmacological targets for ulcer disease. **Conclusion:** This review aims to highlight some of the new and currently used experimental models that are used for the evaluation of gastric and duodenal anti-ulcer and gastric cytoprotective activity of novel anti-ulcer agents.

Key words: Gastric ulcer, duodenal ulcer, ulcer models, prostaglandin, non steroidal anti-inflammatory drugs

INTRODUCTION

The most important undertaking in science is the establishment of experimental methods. Ivan P. Pavlov (1849-1936). Pavlov's statement is readily applicable to peptic ulcer research, i.e., establishing an experimental ulcer model that resembles human ulcers is of utmost importance. Peptic Ulcer Disease (PUD) encompassing gastric and duodenal ulcer is the most prevalent chronic gastrointestinal disorder and is inflammatory in nature (Valle, 2008). The pathophysiology of PUD involves an imbalance between offensive or injurious (acid, pepsin and *Helicobacter pylori*) and defensive mucosal factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors) (Wallace and Sharkey, 2011). Lifetime prevalence of PUD in the United States is approximately 12% in men and 10% in women. Moreover an estimated 15,000 deaths occur each year as a consequence of PUD (Valle, 2008). The control of PUD represents a major triumph for modern pharmacology, Proton Pump Inhibitors (PPI's) are considered superior for acid suppression in most clinically significant acid peptic diseases. Recently, a global market sale of PPI's-only of esomeprazole is 8.4 billion dollars. In India, PUD is common and antacids and antiulcer drugs share 6.2 billion rupees and occupy 4.3% of the Indian Pharmaceutical market share. Today, there are two main approaches for the treatment of PUD. The first deals with the reduction of gastric acid secretion and the second with re-inforcement of gastric mucosal protection. Recently, there has been a rapid progress in the understanding of the pathogenesis of peptic ulcer. Most of the studies focus on newer and better drug therapy. These have been made possible largely by the availability of the proton pump inhibitors, histamine receptor blockers, drugs affecting the mucosal barrier and the prostaglandin analogs.

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These advances in the discovery of novel and more effective anti-ulcer drugs is due to the introduction of a larger number of newer experimental methods to evaluate the anti-ulcer activity of drugs effective in different types of gastro-duodenal ulcers vis-a-vis to study their mechanism of action. Several *in vivo* models of gastric damage have been well characterized and are the primary tools used by gastrointestinal physiologists, pharmacologists and pathologists to study new mechanisms of pathogenesis and new pharmacological targets for ulcer disease. Some of these models that are used for the evaluation of gastric and duodenal anti-ulcer and gastric cytoprotective activity of novel anti-ulcer agents will be reviewed herein that are presently used in order to elucidate their molecular mechanism of action.

Animal models represent an attempt to imitate the pathologies associated with human disease states in a preclinical setting. In using animal models it is therefore important to create a test system that allows the basic mechanism of pathology to be systematically manipulated so as to obtain a better understanding of its biological basis. An important issue in this regard is construct validity-the degree to which the model corresponds to the clinical state in humans. So, in general, experimentally induced gastric and duodenal ulcers should resemble the appearance, complications, development and mode of healing to human clinical ulcers. Nonetheless, although easily stated, developing an experimental method proves really to be difficult. The problems encountered in the experimental evaluation of anti-ulcer drugs result in part from the lack of complete understanding of the physiological and biochemical mechanisms involved in the formation of ulcers. Significant new information has been forthcoming on the pathogenesis of various types of drug induced ulcers and great strides in our basic understanding of gastro-duodenal physiology have been undertaken. Thus, using the experimentally induced models of gastric and duodenal ulcers it is possible to evaluate the therapeutic agents rapidly and with reasonable predictability for their therapeutic usefulness. Nevertheless, certain requirements become necessary in the selection of experimental models for screening anti-ulcer compounds. This includes:

- They should be simple, reproducible and allow for easy quantification of results
- They should make use of a variety of animal species
- They should induce characteristic ulceration in specific locations (stomach and duodenum)
- They should involve different mechanisms by which ulceration is produced
- The ulcers induced should not spontaneously heal during the observation period

Choice, selection, care and preparation of animals: It is mandatory to use a large number of animals to generate dependable and reproducible data on evaluation of anti-ulcer activity of new compounds. This factor restricts the choice to rodents especially the rats and to some extent the guinea pigs. The rat stomach shows an obvious division into two parts: the upper non-secretory portion rumen and the lower glandular secretory portion which is analogous to the body of the stomach in man both anatomically and functionally (Shay *et al*., 1945). The rat being omnivorous resembles man nutritionally. It is advisable to use adult rats of either sex for the anti-ulcer studies. Prior to the experimentation they are usually fasted for a period of 24 h allowing free access to drinking water. Coprophagy has to be carefully avoided in these animals.

We herein discuss several alternate explanations for the cause of gastric ulcers by examining various experimental models of gastric mucosal damage, including stress, ethanol and nonsteroidal anti-inflammatory drug-induced gastric lesions. Further, we review the duodenal antiulcer and chronic gastric ulcers studies and to understand the molecular mechanism of action.

GASTRIC ULCERS

Stress-induced gastric lesions: Psychogenic factors, such as stress, play a major role in the pathogenesis of gastric ulcers in man. Gastrointestinal erosion is one of the consistent findings in man and in experimental animals subjected to different types of stress. It has been known for many years that patients with severe burns, trauma or other serious diseases develop severe intestinal bleeding or perforation caused by ulcers. Endoscopy later revealed that severe physiological stress induces lesions ranging from erosions to complicated ulcers. Clinically, the term stress ulcer encompasses both upper gastrointestinal hemorrhage and lesions as a consequence of trauma including burns, intracranial injuries and septic shock. The first report of the use of restraint as a stress factor was published by Selye (1936). Hanson and Brodie (1960) and Bonfils *et al*. (1966) described methods to study the effect of anti-ulcer drugs on immobilization stress in rats. The method involved prolonged starvation for 36 h and for restraint the animals were placed in a piece of galvanized steel window screen of appropriate size. The screen was moulded around the animal and held in a place with wire staples. To restrain the animal the limbs were put together in pair and tightened with adhesive tape. On examination of the restraint procedure several other co-workers concluded that this technique though useful had several disadvantages. The most important fact was that the lesions did not penetrate the muscularis mucosa

and hence cannot be considered as ulcer in its true sense and also the procedure used has ethical issues. Hence, the restraint method has been modified where the restrained animals were subjected to additional water immersion (water-immersion induced restraint ulcer) (Hayase and Takeuchi, 1986). Many animal models of stress gastric lesions exist but the most widely used and reliable model is that of immobilization or restraint along with exposure to cold conditions (Vincent *et al*., 1977). Exposure of rats to cold conditions during the restraint period accelerates the occurrence of gastric ulcers and shortens the time of necessary immobilization (Takagi *et al*., 1964; West, 1982). They bring central nervous system into play and the lesions produced by these methods are located in the glandular portion region of the stomach. This procedure is simple, effective and produced a reliably high incidence of gastric glandular lesions. More importantly in most cases, these lesions penetrate the muscularis mucosa and as such may be called ulcers. This experimental model resembles the psychogenic factors in the pathogenesis of gastric ulcers in patients. Thus, stress-related animal experiments appear to be a very good mimic of the human condition and have allowed studies into pathogenic mechanisms as well as useful therapeutic interventions.

Pyloric ligation induced ulcer model: Gastric acid plays a major role in the pathogenesis of gastric and duodenal ulcers (Hunt *et al*., 1995). In the past, the long held clinical dictum no acid-no ulcer led to excessive focus on gastric acid secretion as a causative factor in ulcer disease resulted in a preponderance of research effort being directed toward endogenous aggressive factors as the major sources of and hence targets for the treatment of, gastric ulcers. Gastric acid secretion is mediated by the enzyme H+, K+-ATPase or proton pump localized on the luminal membrane of the parietal cells (Saccomani *et al*., 1979; Sachs, 1988; Sachs *et al*., 1976). Therapies against ulcers, therefore, either neutralize or reduce acid secretion through use of antacids (Goan, 1989), histamine H2 receptor antagonists (Black *et al*., 1972; Brimblecombe *et al*., 1975), or proton pump inhibitors (Kakei *et al*., 1993; Lindberg *et al*., 1990; Nagaya *et al*., 1990).

A simple and reliable method for induction of gastric ulceration in rats, based on ligature of the pylorus, has been published by Shay *et al*. (1945). The ulceration is caused by accumulation of acidic gastric juice in the stomach. Apart from the microscopic observation of the lesions formed, the accumulated gastric juice is also collected for the estimation of gastric free and total acidity, mucin and pepsin activity studies.

Gastric secretion study: Free and total acidity was measured from the collected gastric juice by titrating against 0.01 N NaOH, using phenolphthalein as an indicator and expressed in terms of μequiv./mL.

Luminal pepsin results in the proteolytic digestion of the underlying gastric epithelium. So peptic activity was determined by measuring the amount of liberated tyrosine by the action of pepsin on hemoglobin as substrate (Debnath *et al*., 1974).

Secretion of mucus gel results in the formation of a barrier to luminal pepsin, thereby protecting the underlying mucosa from proteolytic digestion. In order to identify the mucoprotective effects of the test substances, the mucus content can also be estimated (Varley *et al*., 1980). Thus, this model has been proved to be a valuable tool to evaluate anti-ulcer drugs with mechanisms of actions, both anti-secretory and mucoprotective effects.

NSAID-induced gastric lesions: Despite their well-accepted anti-inflammatory and analgesic benefits, gastric mucosal damage as a result of treatment with nonsteroidal anti-inflammatory drugs (NSAID) is recognized as the most serious adverse reaction to this class of compounds (Schoen and Vender, 1989) and has been the stimulus behind much of the research in the past decade aimed at developing more effective gastroprotective compounds. NSAIDs, including aspirin, significantly increase the risk of adverse gastrointestinal events, particularly those related to gastric and/or duodenal mucosal injury: erosions, ulcers and ulcer complications, especially bleeding (Laine, 2001). About 15-30% of regular NSAID users have one or more ulcers when examined endoscopically and 3-4.5% of NSAID users have clinically significant upper gastrointestinal events, including ulcers and ulcer complications (Yuan *et al*., 2006). Patients taking low-dose aspirin for the prevention of a cardiovascular event, such as myocardial infarction or thrombotic stroke, are also at increased risk of gastrointestinal injury and complications (Weisman and Graham, 2002). The damage is caused mainly through the ability of these agents to inhibit prostaglandin synthesis which has a negative impact on several components of mucosal defence. The injurious gastrointestinal effects of NSAIDs are largely caused by the inhibition of COX and its role in normal mucosal defense mechanisms and also through the inhibition of thromboxane A2 which compromises platelet function and results in gastrointestinal bleeding.

Parenteral administration of a variety of NSAID, including indomethacin and acetylsalicylic acid, represents a very simple and effective animal model for studying the mechanisms underlying NSAID-induced gastropathy. NSAID induced gastric damage is mediated

through the well-characterized effect of NSAID to block cyclooxygenase and thereby inhibit endogenous prostaglandin production (Schoen and Vender, 1989). Dose-and time dependency of the ulcerogenic actions of indomethacin were studied by Djahanguiri (1969). Instead of indomethacin, gastric lesions can be induced by intravenous or oral doses of aspirin that can be prevented by exogenous PGE2 or PGI2 (Konturek *et al*., 1981).

Estimation of Prostaglandin (PG) generation: Animal studies thus have played an important role in identifying the major mechanism of the systemic impact of NSAIDs, namely the reduction of mucosal prostaglandin production. Prostaglandins play a central role in gastric epithelial defense/repair. Every component of mucosal defence is to some extent prostaglandin-dependent, so inhibition of prostaglandin synthesis by NSAIDs leads to an increased susceptibility of the stomach and duodenum to injury induced by luminal irritants. Moreover, suppression of prostaglandin synthesis leads to marked alterations in the microcirculation of the stomach and intestine that appear to be critical and to early events in the pathogenesis of ulceration. Suppression of prostaglandin synthesis can also have a negative impact upon the secretion of mucus and bicarbonate by the gastric and duodenal epithelium, the proliferation of epithelial cells. Thus, estimation of PG generation is of immense importance in context to NSAID induced gastropathy and compound which results in the improvement of PG generation indicates to the cytoprotective activity of the compound.

Ethanol induced ulcer and gastric cytoprotection: The concept of gastric cytoprotection signifies protection against mucosal injury by a mechanism other than inhibition of acid secretion was introduced long ago (Robert *et al*., 1979; Vogel, 2008). Since then cytoprotection against various necrotizing agents has been routinely used to assess the anti-ulcer potential of different compounds. The ethanol-induced acute gastric mucosal injury model is considered to be one of the widely used experimental models of ulcer disease. Ethanol, being a necrotizing agent, damages the superficial epithelial layers and inhibits the release of mucosal prostaglandins (Miller and Henagan, 1984).

Intragastric application of absolute ethanol is a reproducible method to produce gastric lesions in experimental animals (Robert *et al*., 1979; Szabo *et al*., 1981). Gastric lesions can be observed only after an hour of administration of alcohol. In general, severity of mucosal involvement has been assessed by counting the number of haemorrhagic spots or by scoring the mucosal lesions but alcohol induced lesions appear as blackish lesions grouped in patches of varying size, usually parallel to the major axis of the stomach. Witt *et al*. (1985) described a method to objectively quantify the extent of ethanol-induced gastric lesions utilizing a transmission densitometer to measure the optical density of the photographic negative of the stomach mucosa. The method has been modified by several authors. Presently, a microprocessor linked image analyzing software with a trinocular zoom stereomicroscope is used to measure the area of damaged mucosa and the length of the lesions.

These lesions can be at least partially inhibited by various drugs, such as some prostaglandins. The protective effect against various irritants has been called cytoprotective activity (Robert, 1979). Thus an agent that shows protection against ulcer induced in this model, might be cytoprotective in nature and exerts this protection by stimulating the release of endogenous prostaglandins and mucus secretion in the gastric mucosa.

Acetic acid induced chronic gastric ulcer: Of the various problems relating to human peptic ulcer disease, one of the least understood aspects is the chronicity of the disease that is characterized by repeated episodes of healing and re-exacerbation, a phenomenon which is problematic to both patient and clinician. Most experimental ulcerative lesions discussed above thus far heal quickly in a few days without scar formation and do not re-ulcerate spontaneously. Takagi *et al*. (1969) developed a model for inducing chronic gastric ulcer in rats by means of submucosal injection of acetic acid and reported on the healing process of lesions for extended intervals after the ulcer preparation. The experimental gastric ulcer was termed chronic because it persisted for a long time and resembled human chronic ulcer both grossly and histologically. Since its development in 1969, modifications were made to acetic acid induced ulcer in order to circumvent certain drawbacks such as, consistent adherence of ulcer base to the adjacent organs (mainly liver). A new method was developed by Okabe which involves intraluminal application of acetic acid solution (Okabe and Pfeiffer, 1971). The model has become well established and is now used throughout the world by basic and clinical scientists.

Animals are deprived of food for 24 h before the experiments but were provided with drinking water *ad libitum*. Under pentobarbital anesthesia, abdomen is incised and stomach is exposed. The anterior and posterior walls of the stomach are clamped together with

a forcep armed with a round ring (9 mm ID). 40% acetic acid (0.2 mL) is injected into the clamped portion of the stomach with an 18 gauze needle. Acetic acid solution is injected into the clamped portion through the distal antrum. Forty five sec later, the acid is removed and the abdomen is closed. As expected, two deep, round ulcers, one on the anterior wall and the other on the posterior wall, developed in the area that has been exposed to the acetic acid solution. Clearly defined deep ulcers consistently developed after 3 days of acid application and the respective treatments are started from this day onwards and continued for a period of 10 days. Sham-operated rats are subjected to the same surgical procedure without application of acetic acid.

The reasons underlying the model's frequent use as the chronic ulcer model of choice appear to be as follows:

- The ulcer induction procedure is quite simple, readily resulting in ulcers of consistent size and severity at an incidence of 100%
- The ulcer models highly resemble human ulcers in terms of both pathological features and healing mechanisms. Indeed, spontaneous relapse of healed ulcers is frequently observed, just as in peptic ulcer patients
- The ulcers respond well to various anti-ulcer drugs, such as acid pump inhibitors, histamine receptor antagonists and sucralfate
- Moreover, both steroidal and non-steroidal anti-inflammatory drugs negatively impact healing of the experimental ulcers

Thus this chronic ulcer model is now used as the standard model for screening compounds as potential anti-ulcer drugs.

DUODENAL ULCER

Peptic ulcer encompasses both gastric and duodenal ulcer. Understanding the pathophysiology of peptic ulcer disease is sometimes difficult as though they share common features in terms of pathogenesis, diagnosis and treatment, mechanisms of injury differ distinctly between duodenal and gastric ulcers. Duodenal ulcer is caused mainly by an increase in acid and pepsin load and gastric metaplasia in the duodenal cap.

Histamine induced duodenal ulcer in guinea pigs: Experimental duodenal ulcers in rats induced by cysteamine HCl were first described by Selye and Szabo (1973). Cysteamine induced duodenal ulcer in rat has been widely used as a model of peptic ulcer disease. This chemically induced ulcer resembles duodenal ulcer in man to its location, histopathology and some aspects of pathophysiology. Cysteamine stimulates gastric acid secretion rate and inhibits the alkaline mucus secretion from the Brunner's glands in the proximal duodenum resulting in the formation of duodenal ulcer.

Duodenal ulcers due to administration of cysteamine develop in the anterior and posterior wall of the proximal duodenum. The more severe ulcers, located on the anterior wall, frequently perforated, resulting in focal or generalized peritonitis, or penetrated into the liver whereas, the one on the posterior wall penetrated into the pancreas. These drawbacks limited the use of this model further in a drug screening programme.

Later, intramuscular application of histamine was used to induce duodenal ulcer in guineapigs as it is known that rats are resistant to induction of duodenal ulcers by histamine and so guinea pigs are the animals of choice for inducing duodenal ulcers as they are highly sensitive to histamine. Duodenal ulcers are induced in guinea pigs by intramuscular application of histamine acid phosphate at a dose of 0.25 mg kg^{-1} at every 30 min interval for 4 h and the animals are sacrificed after 30 min of the last dose (Cho and Pfeiffer, 1981). Animals are treated with the test drug and the standard drug omeprazole (10 mg kg^{-1}, p.o.) was administered 45 min prior to histamine administration. Promethazine hydrochloride at a dose of 2.5 mg kg^{-1} of body weight is injected intraperitoneally to each animal 15 min prior to administration of histamine, in order to protect the animals from histamine toxicity.

ASSESSMENT OF THE EXTENT OF EXPERIMENTAL EROSIONS AND ULCERS

The quantitative assessment of experimentally induced gastric and duodenal ulcers is done through various scoring systems which have been developed to quantitate gastric ulceration by calculating ulcer indices. A number of methods and indices have been used to score the extent of ulceration on arbitrary scales which are highly subjective and hence must be carried on blindly by two trained observers unaware of the experimental protocols. Severity of ulcers is scored with a stereo-zoom microscope using the following arbitrary scoring system as described by Srivastava *et al*. (1991):

- Shedding of epithelium = 10
- Petechial and frank hemorrhages = 20
- One or two ulcers = 30
- More than two ulcers = 40
- Perforated ulcers = 50

Evaluation: An ulcer index UI is calculated:

$$UI = UN+US+UP\times10-1$$

Where:
UN = Average of number of ulcers per animal
US = Average of severity score
UP = Percentage of animals with ulcers

Percentage protection is calculated as:

$$\text{Percentage protection} = \frac{C-T}{C}\times100$$

Where:
C = Mean severity of ulcer score in control group
T = Mean severity of ulcer score in treated group

ESTIMATION OF PROTON PUMP (H+ K+-ATPASE) ACTIVITY

In order to evaluate the antisecretory potential of anti-ulcer compounds, the inhibitory effect of the compound on enzymatic activity of proton pump (H+K+ATPase) is estimated using gastric microsomes isolated according to Berglindh, from nonstimulated rat stomach by measuring inorganic phosphate released from the gastric microsomes (Berglindh, 1990). For the enzyme assay, gastric microsomes, previously incubated with or without different concentrations of drug and standard anti-ulcer drug, omeprazole for 10 min at 37°C were added to an assay buffer and the reaction was carried out at 37°C for 20 min and was stopped by adding 10% ice-cold trichloroacetic acid. After centrifugation (2000 g for 1 min), inorganic phosphate release was determined spectrophotometrically at 310 nm wavelength (Sanui, 1974). Nonspecific ATPase activity was subtracted from the estimated H^+K^+ATPase activity and the IC_{50} value of the test compound is calculated.

ELUCIDATION OF MOLECULAR MECHANISM OF ACTION THROUGH EXPRESSION ANALYSIS STUDIES

Role of growth factors in chronic ulcer healing: Ulcer-healing is a complex process that depends on regeneration of mucosal glandular structure and migration of epithelial cells to cover ulcer crater. Results from animal studies are in agreement with clinical impressions regarding the importance of rapid spontaneous healing and suggest that the following growth factors play an important role:

- Epidermal Growth Factor (EGF)
- Basic Fibroblast Growth Factor (bFGF)
- Platelet Derived Growth Factor (PDGF) and
- Vascular Endothelial Growth Factor (VEGF)

Growth factors such as EGF, PDGF and bFGF activate epithelial cell migration and proliferation and accelerate ulcer healing in vivo, whereas, the growth of granulation tissue and formation of new microvessels through angiogenesis is stimulated by VEGF. Thus, in order to understand the ulcer healing activity of the test compounds, alterations in the levels of protein expression of these growth factors are observed by Western Blot analysis.

Briefly, normal gastric tissue from sham-operated rats and gastric ulcer specimens control and treated group are lysed in lysis buffer using Polytron homogenizer. The lysate is clarified by centrifugation at 14,000 rpm for 10 min. Equal amounts of protein from the lysates are subjected to separation on SDS-PAGE and immunoblotted to nitrocellulose membranes. Further, membrane is probed with corresponding primary and secondary antibodies. Thereafter, chemiluminescence is detected and the immunoreactive area is determined by densitometric analysis using Biovis gel documentation software.

Modulation of inflammatory responses during gastric ulcer healing: In chronic gastric ulcer there is disruption of the mucosal integrity leading to a local defect or excavation due to active inflammation. Thus healing of chronic ulceration not only requires cell migration, proliferation and angiogenesis but also amelioration of active inflammation. Inflammation of the mucosal layer is a feature almost always associated with ulceration of gastric tissues. Interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) are the major proinflammatory cytokines and IL-10, the anti-inflammatory cytokine that play an important role in the inflammatory responses accompanied with chronic ulceration of gastric mucosa. Furthermore, Cyclooxygenase (COX-2) is an important rate limiting enzyme involved in inflammatory processes due to its role in the production of prostaglandins from arachidonic acid. Thus, effects of the test compounds are observed on the alterations in the levels of gene expression of these inflammatory mediators by Reverse transcriptase Polymerase Chain reaction (RT-PCR).

Briefly, total RNA was extracted from gastric mucosal samples using TRIZOL Reagent (Invitrogen Life Technologies, Germany) and cDNA was generated from extracted RNA using RETROscript kit (Ambion Inc, USA.) following manufacturer's instructions. Genes for TNF-α, IL-1β, IL-10, COX-2 and β-Actin are amplified with specific primer sets. Intensity of the PCR products is measured using Biovis gel documentation software and expressed in terms of relative intensity of PCR-product/β-Actin ratio.

HELICOBACTER PYLORI INFECTION MODEL IN MICE

Marchetti *et al.* (1995) and Konturek *et al.* (1999) described a mouse model of *Helicobacter pylori* infection (Marchetti *et al.*, 1995; Konturek *et al.*, 1999). Gastric function and healing of chronic acetic acid-induced ulcers in BALB/c mice were studied after inoculation with CagA and VacA positive (type I) or CagA and VacA negative (type II) *Helicobacter pylori* strains. This infection caused immediate suppression of gastric secretion and delayed the healing of ulcers.

CONCLUSION

Over the years, an increasing number of studies have been undertaken to strive towards both clarification of the mechanisms underlying ulcer healing and discovery of additional anti-ulcer pharmaceuticals. In particular, several animal models of gastric mucosal injury and protection have been devised and used for screening of anti-ulcer drugs and elucidation of pathogenesis. Information derived from these models suggests that mechanisms other than gastric acid secretion are involved in the pathogenesis of gastric ulcer disease and should therefore increasingly occupy the focus of pharmacological investigation. Elucidation of the complete time course of pathological events that precede the development of chronic gastric ulcer is an important direction for the future and is likely to yield significant therapeutic information. Acetic acid induced ulcer models appear to be incredibly useful for pathophysiological and pharmacological studies of peptic ulcers. With the use of acetic acid ulcer models, a new anti-ulcer drug that prevents ulcer relapse, as well as enhances ulcer healing, could potentially be developed. Furthermore, utilizing molecular biology techniques, to include gene therapy, it is now possible to more precisely analyze the mechanisms underlying ulcer healing, expediting discovery of more effective ulcer therapy. In recent years, genetically modified mice such as, H2-receptor knockout and gastrin-transgenic mice allowed rapid accumulation of very important findings.

Nevertheless, certain aspects should be considered during the development of experimental models for screening anti-ulcer compounds. This include, that the method produces a consistently high incidence of readily discernible ulcers in a definite area of the gastrointestinal tract and that either the production or healing of these ulcers can be modified by known therapeutic agents. Furthermore, the appearance, complications, development and healing of these experimentally induced ulcers must resemble human clinical ulcers. Thus, these experimental ulcer models appear to lend themselves to the screening of therapeutic drugs for peptic ulcers of the stomach or duodenum and to the investigation of the mechanisms of chronic ulcer.

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