Research Article

Effect of Indazole and Its Derivative on Aspirin Induced Gastric Ulcers in Rats

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ABSTRACT

Peptic ulcer is the very common disease affecting millions of people in the world. Gastric hyper acidity and ulceration of the stomach mucosa due to various factors are serious health problems of global concern. Several drugs widely used to prevent gastro intestinal ailments, which include H_2 receptor antagonist and Proton pump inhibitors. Due to problems associated with current treatment, there is the need to seek alternative drug source against gastric ulcers. Hence, the present study was undertaken to determine the anti-ulcer property of *Indazole, 5-Aminoindazole and 6-Nitroindazole* against aspirin induced gastric mucosal injury in rats. Anti-ulcer activity was evaluated by measuring the ulcer index and Histopathological investigations. Results of this study showed that pre-treatment with Indazole and its derivatives provided significant protection against gastric ulcer. The gastro-protective effect of indazoles may be due to inhibition of cytokines and free radical, which are reported in our previous study.

Keywords: Anti- ulcer, Indazoles, Aspirin induced ulcer.

INTRODUCTION

Gastric hyper acidity and ulceration of the stomach mucosa due to factors are serious health problems and is a worldwide problem. Peptic ulcer is a benign lesion of gastric or duodenal mucosa occurring at the site where the mucosal epithelium is exposed to acid and pepsin.[1,14] Peptic ulcer disease induced by several factors including stress, nutritional deficiencies, NSAIDS and the disease affects a large portion of the world population.[2] Synthetic NSAID´S like aspirin cause mucosal damage by interfering with PG synthesis, increasing acid secretion and back diffusion of H^+ ions and thus leading to breaking up of mucosal barrier.[3] Now a days, the studies first deals with reducing the release of gastric acid and the second with reinforcing gastric mucosal protection.[4,5] Although a number of anti-ulcer drugs are available, all these drugs have side effects and limitations.

In general Peptic ulcer caused by imbalance between the "aggressive" and "defensive" factors. The aggressive factors include acid, pepsin, free radicals, infectious agents like *Helicobacter pylori*, chemicals and to a lesser extent bile salts and pancreatic enzymes. While the defensive factors include the mucin, bicarbonate, prostaglandins and mucosa blood flow.

Due to any cause, an increase in aggressive factors or a decrease in defensive factors will lead to loss of mucosal integrity resulting in ulceration.[6] The etiological factors are: diet,[7] alcohol consumption,[8] smoking,[9] non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, naproxen and ibuprofen,[10] psychological stress,[11] corticosteroids,[12] *H. pylori* infection[13] and genetic factors,[14] and also adult males being predominantly affect duodenal ulcer.[15] The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence.

Non-steroidal anti-inflammatory drug (NSAID) like aspirin is used for the treatment of rheumatoid arthritis, many inflammatory and related diseases as well as the prevention of cardiovascular thrombotic diseases. Gastric ulcer associated with the use of aspirin is a major problem. Many factors such as gastric acid and pepsin secretion, gastric microcirculation, prostaglandin E2(PGE2),[16] and pro-inflammatory cytokines like interleukin(IL)-1 and tumor necrosis factor (TNF)[17] play an important roles in the genesis of gastric mucosal damage, and its subsequent development[18] it has been reported that increases in NO synthase (NOS) activity is involved in the gastrointestinal mucosal defence and also in the pathogenesis of mucosal damage.[19] Very limited studies has been conducted on Indazole derivatives in ulcer study.

The present study; pathological investigations on Indazole and its derivatives in gastric mucosal damage induced by aspirin. Gastric mucosa significantly protected co-administration of Indazoles with aspirin induced ulcer.

MATERIALS AND METHODS

Experimental Animals

Adult Wister rates of male weighing 100-150g were used in the present study and these were purchased from the Kings Institute of preventive medicine, Gundy. The animals had free access to food and water and maintained at 24±1 °C temperature with 12h day/12h

night cycle. All the experiments were carried out between 09.00 and 13.00 hours to avoid circadian variation.

The experiment was carried out in Sree Balaji Medical College and Hospital, Chennai.

The experimental protocol was approved by the institutional animal ethical committee (002/02/IAEC/2014/SBMCH).

In all the experimental studies each group consisted of six animals.

Effect of Indazoles on Gastric Mucosa[22,23]

The different indazoles were administered in a dose of 100mg/kg orally to male wistar rats for three consecutive days.

The animals were sacrificed five hours after indazole treatment on the third day and the stomach was exposed, opened along the greater curvature and examined with a magnifying glass for the presence of ulcers (The number of ulcer spots in the glandular portion of the stomach were counted in both control and drug treated animals and the ulcer score was calculated) histopathological sections observed a group of animals treated with carboxy methyl cellulose (1% CMC) served as control.

Effect of Indazoles on Aspirin Induced Gastric Ulcer[20-22]

The different indazoles were administered in a dose of 100mg/kg orally to wistar rats for three consecutive days and followed by after 12 hours fasting period, aspirin (100 mg/kg) was administered orally to all the above treatment groups.

The animals were sacrificed five hours later and the stomach was excised.

It was opened along the greater curvature and ulcer was scored according to the severity and observed histopathological sections, (The number of ulcer spots in the glandular portion of the stomach were counted in both control and drug with aspirin treated animals and the ulcer score was calculated).

Experimental Animal Treated Groups

Group-I

1%CMC (Control)

Group-II

Aspirin alone (100mg/kg)

Group-III

indazole (100mg/kg)

Group-IV

5-aminoindazole (100mg/kg)

Group-V

6-nitroindazole (100mg/kg)

Group-VI

Indazole (100mg/kg) **+** aspirin (100mg/kg)

Group-VII

5-aminoindazole (100mg/kg) **+** aspirin (100mg/kg)

Group-VIII

6-nitroindazole (100 mg/kg) **+** aspirin (100 mg/kg).

RESULTS

Ulcer Score

The ulcerative score was calculated by severity of gastric mucosal lesions and graded as follows;

Erosions Score was 0 treated with control group, Score was 2 treated with aspirin alone, Score was 0 treated with all tested Indazoles and score was 0.5 treated with aspirin with all tested indazoles.

The Histopathological Findings of Gastric Mucosa

The gastric mucosa obtained from the control group (Fig.1) was shown normal cellular architecture (not shown gastric ulcer) and damaged mucosal epithelium and cellular debris were found in rats stomachs treated with aspirin (A) alone (Fig.2); however, the histological examination of gastric mucosal epithelium treated with Indazole(I),5-aminoindazole(5-AI),6-nitroindazole(6-NI) (Fig. 3,4,5) showed normal cellular architecture and no pathological changes (ulcer not shown), and all tested indazoles are protected gastric mucosa against aspirin treated rates [shown less gastric ulcer treated with aspirin+ indazole (A+I,Fig.6), aspirin+5-aminoindazole(A+5-AI, Fig.7) and aspirin+6-nitroindazole (A+6-NI, Fig.8)].

DISCUSSION

In the present study, the anti-ulcerative effects of Indazole and its derivatives were investigated in aspirin-induced gastric ulcer model rats. Aspirin has been reported to reduce the gastric juice pH and increase the volume of gastric juice.[40]

In the present study, gastric lesions and epithelium damage induced by aspirin and recovered by the co-administration of aspirin and Indazole and its derivatives protective effect on gastric mucosa may be by inhibition of cytokines, Nitric oxide production and due to varies mechanisms involved.

The mechanism underlying, Inflammation and neutrophil infiltration are also important role in pathogenesis of the gastric damage induced by NSAIDs.[24-26]

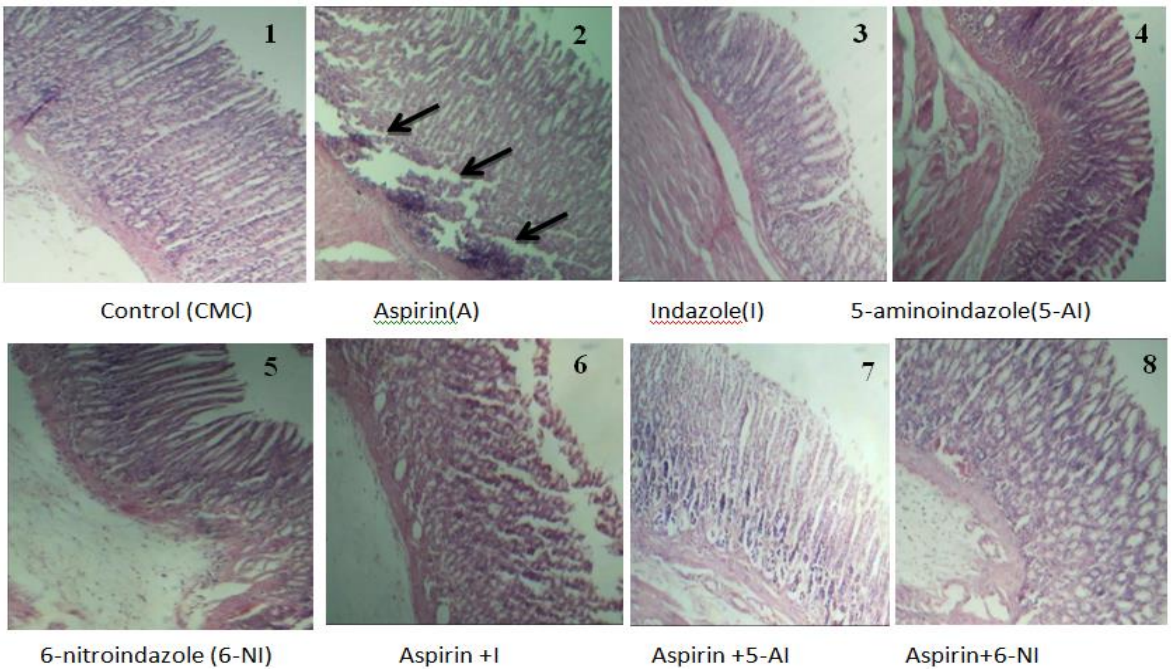
The inflammation induced in the gastric mucosa by aspirin is accompanied by increased TNF- production,[27,28] which augments neutrophil derived superoxide generation[29] and stimulates IL-1 production, leading to neutrophil accumulation.[30,31] The previous studies Indazole, 5-aminoindazole, 6-nitroindazole are significantly inhibited TNF-α, IL-1β, LPO in in-vitro

models[32] may be protect gastric mucosa by inhibiting following cytokines.

Another mechanism Nitric oxide (NO) is a mediator and not only of GI mucosal defence,[33] but also of its damage the mucosa.[34] One of the mechanisms by which aspirin damages the gastric mucosa and increased production of NO due to the overexpression of iNOS.[35] It has been shown that different concentrations of NO have completely opposite effects in the same tissue.[36] In general, the mucosal and endothelial NOS isoforms produce low amounts of NO. However, the high quantity of NO produced by iNOS damages the epithelium.[36,37] The excessive release of NO from gastric epithelial cells induced by aspirin has been reported to exert detrimental effects.[38,39] In the present study, may be Indazole and its derivatives reduced iNOS activity and inhibited the production of gastric ulcers, even in the presence of aspirin.

Prostaglandins have protective effects against various gastric injury models.[41] Aspirin has been shown to reduce the mucosal PGE_2 content[40,42] by inhibition of COX enzymes (COX-1 and COX-2).[43] The lack of attenuation of the decrease in gastric mucosal PGE_2 content after the co-administration of Indazole and its derivatives also reveals that the restoration of the PGE_2 level in the gastric mucosa is not the mechanism underlying the protective effects of indazoles in this aspirin induced ulcer model. This is not unexpected because the reduction of the gastric mucosal PGE_2 concentration induced by aspirin does not necessarily participate in gastric ulcer generation.[42,44] But all the present tested compounds inhibition of COX-2 activity.[32] Aspirin are known to induce gastric damage by inhibits of prostaglandins and play a important protective role, stimulating the secretion of bicarbonate and mucus maintaining mucosal blood and regulating mucosal turn over and repair in the stomach and oxy radicals may also play important role in the aspirin induced erosive gastritis[44].

Mechanism by increasing mucus production, anti-oxidants play a major role in repairing the gastric damage.[46] Reactive Oxygen Species (ROS) are continuously produced during normal physiologic events and removed by anti-oxidant defence mechanism.[47] In pathological conditions, ROS are over produced and result in lipid peroxidation and oxidative damage. The imbalance between ROS and anti-oxidant defense mechanisms leads to oxidative modification in the cellular membrane or intracellular molecules.[48] In the present study, administration of aspirin with Indazoles marked reduction of gastric mucosal damage may due to marked reduction of oxidants and lipid peroxidation in in-vitro studies.[32]



CONCLUSION

The present study reveal that, Indazole and its derivatives is suggested to protect the stomach against the ulcer formation induced by aspirin may be due to reducing iNOS activity and inflammatory cytokine (TNF and IL-1) expression, anti-oxidant property in gastric mucosa in previous studies.

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