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Original Research Article

Peptic Ulcer aetiology; role of H, K-ATPase enzyme and biochemical mediators in a reserpine induced ulcer model

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Abstract

Gastric ulcers of the stomach are a serious disorder which if untreated could lead to some times life threating consequences. In hypergastrinemic states such as Zollinger-Ellison syndrome, or where acid secretion has to be inhibited by more than 20% over a 24-hr period, such as for treatment of esophagitis, NSAID damage, or gastric ulcers, the dose and frequency of administration of the currently available antagonists must be increased to achieve curative therapy. This has opened the avenues to search for an alternative target for acid inhibitory drugs, such as the gastric acid pump, the H, K-ATPase enzyme. This article focuses on the function of this ATPase and suggests that inhibition of this pump will provide a more efficacious means of reduction of acid secretion by the stomach, hence improving and simplifying therapy of acid related diseases. It also examines the effect of PUFA containing oils and conventional antacid drugs on the biochemical parameters in the reserpine induced ulcers in the rat.

Keywords: H, K-ATPase, Gastric, Phospholipid, Ulcer, NSAID.

1. Introduction

The peptic ulcer disease may lead to upper gastrointestinal haemorrhage and perforation, which may have high morbidity and mortality rates. In the majority of cases, *H. pylori* bacteria increase the production of reactive oxygen species [1]. Non-steroidal anti-inflammatory drugs (NSAID) can cause submucosal erosion and inhibit cyclooxygenase, which reduces the formation of prostaglandins and weakens the protection by the gastric mucosal layer [2]. In spite of the multifaceted pathogenesis of peptic ulcers, increased secretion of gastric acid is still recognized as a critical aetiological factor of this disease [3][4]. Therefore, the main therapeutic target is to control acid secretion using antacids, H_2 receptor blockers (ranitidine and famotidine), or proton pump inhibitors (omeprazole and lansoprazole).The combination of antacids, antisecretory drugs, and antimicrobial agents has been in use for peptic ulcer treatment [5].

There is increasing evidence that the gastric proton pump i.e H,K-ATPase may be interacting with chloride channels and possible mediators of the phospholipid breakdown. Gastric H,K-ATPase (proton pump) is the transmembrane (TM) enzyme responsible for the creation of the low pH environment in the stomach through an active, electroneutral exchange of cytoplasmic protons for luminal K^+.[6] The H,K-ATPase enzyme consists of a catalytic α-subunit that contains the ATP, inhibitor, and ion-binding sites, and a smaller, glycosylated β-subunit that provides structural stability and participates in membrane targeting[7]. The α-subunit has 62% sequence identity with the Na-K-ATPase catalytic subunit and 29% sequence identity with srCa-ATPase, which lacks a β-subunit. Several factors play a role in gastric acid and pepsin secretion, gastric microcirculation, prostaglandin E2 (PGE2) content and pro-inflammatory duodenal ulcer is the most prevalent gastrointestinal ulcer in which the cytokines interleukin (IL)-1 and tumor necrosis factor contribute. The patho-physiology of ulcer involves the TNF and other mediators and this interplay is responsible in genesis apart from increased acid pepsin, *H.Pylori* and NSAID and the causative agents. It has been reported that increases in NO synthase (NOS) is a defensive factors (mucin, prostaglandin, bicarbonate levels) and its

activity is involved in the maintenance of gastrointestinal mucosal integrity.

There is considerable emphasis on herbal and nutraceutical based therapy of peptic/gastric ulcer like the fish oil and Arasco oil [8]. This study was undertaken to evaluate the influence of PUFA containing oils and antiulcer drugs on biochemical parameters in a reserpine induced model of gastric ulcer.

2. Methods

2.1 Animals used

Wistar albino rats of either sex weighing between 250- 300 g were utilized for this study. The animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at temperature of 24 ± 2°C and relative humidity of 30-70%. A 12:12 dark: light cycle was followed during the experiments. All the animals were allowed to free access to water *ad libitum* and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd., Mumbai). All the experimental procedures and protocols used in this study were reviewed by the institutional animal ethical committee and were in accordance with the guidelines of the CPCSEA.

2.2 Drugs and chemicals

Fish oil - an n3 rich oil Maxepa (EPA & DHA) was procured from Merck, India and Arachidonic acid – rich in n6 from the Cayman chemical, USA were used as source of PUFAs. Omeprazole (OMEZ) Sigma, USA and ranitidine were suspended in 1% Sodium Carboxy Methyl Cellulose (SCMC) and administered to the animals for anti-ulcer studies. All drugs are administered orally. Fish oil – an n3 rich oil and Arasco - rich in n6 AA were used as source of PUFAs. All drugs were administered orally.

2.3 Induction of Gastric Ulcers in rats

In order to produce gastric lesions, 10 mg/kg dose of reserpine (dissolved in few drops of glacial acetic acid and then diluted with distilled water) was administered intraperitoneally (i.p.) according to the method of Gupta *et al.*[9] All drugs are administered orally 30-45 minutes before reserpine treatment. After 20 hrs, animals were sacrificed and their stomach were removed for the evaluation of microscopic and biochemical parameters.

2.4 Experimental Protocol

Six groups of animals were employed in the present study and each group comprised of 6 rats. Fish oil – an n3 rich oil and Arasco - rich in n6 AA were used as source of PUFAs. Anti-ulcer drugs, ranitidine and omeprazole (OMEZ) were suspended in 1% Sodium Carboxy Methyl Cellulose (SCMC) and administered to the animals for anti-ulcer studies. All drugs were administered orally. Reserpine was dissolved in few drops of glacial acetic acid and final volume made up with distilled water and was injected intraperitoneally.

Group I (Normal Control): Rats were maintained on standard food and water and no treatment was given.

Group II (Reserpine Control): Rats were administered single dose of reserpine (10 mg/kg, i.p).

Group III (Omeprazole positive control): Rats were treated with Omeprazole (20 mg/kg, p.o.),

Group IV [Ranitidine (30 mg/kg) treated reserpine]: Rats administered Ranitidine (30 mg/kg, p.o.) were treated with 30 min prior to reserpine administration.

Group V [Fish oil µl/day) for 10 days treated reserpine]: Rats administered reserpine (10 mg/kg, i.p.) were treated Fish oil µl/day and the treatment was administered 30 min prior to reserpine administration.

Group VI [AA µl/day) for 10 days treated reserpine]: Rats administered reserpine (10 mg/kg, i.p.) were treated AA µl/day and the treatment was administered 30 min prior to reserpine administration.

2.5 Determination of free acidity

The free acidity was calculated as per previously shown formula [10].

1. Gastric juice (1 ml) was taken in to a 100 ml conical flask, to this 2-3 drops of Topfer's reagent was added and titrated with 0.01 NaOH until all traces of red colour disappears and the colour of the solution turns yellowish orange (end point).
2. The volume of alkali added was noted. This volume corresponds to free acidity.
3. 2-3 drops of phenolphthalein solution were added and titration was continued until a defined red tinge reappears.
4. The volume of alkali added was noted which corresponds to total acidity. Acidity was calculated by using the formula:

Acidity (mEq/Litre) = Volume of NaOH x Normality of NaOH x 100/0.1 gm

2.6 Estimation of H, K-ATPase activity

The H,K-ATPase activity was assayed in induced ulcer animals based on the techniques of Nagaya *et al*[11]. The assay medium consisted of 70 mM Tris buffer, pH 6.8, 5 mM MgCl2 and the enzyme solution in the presence of 10 mM KCl in a total volume of 1 ml. The reaction was initiated by adding 2 mM ATP, incubated at 37 ◦C for 20 min and the reaction was stopped by 10% TCA. After centrifugation, 2.5 ml of ammonium molybdate and 0.5 ml of l-amino-2-naphthol-4-sulphonic acid were added to the supernatant and the absorbance was read at 620 nm. Results are expressed as mmol of Pi liberated/min/mg protein.

2.7 Measurement of mucosal PGE_2

Frozen gastric mucosal tissue (1 g) was added to 5 ml homogenisation buffer and homogenized. The lysate was then centrifuged in a micro centrifuge at 16,000xrpm for 15 min at 20°C to 80°C. The supernatant was then transferred to a new tube, and its total protein content was analysed by using protein assay. PGE_2 concentrations were investigated using PGE_2 ELISA Kit.

2.8 Determination of iNOS activity

Individual specimens of the gastric mucosa were homogenized in sample buffer containing 10mM EDTA and centrifuged at 13,000 rpm at 4°C for 5min. The supernatant was transferred to a new tube, and its total protein content of 10µg/µL. Gastric mucosal iNOS activity was measured with the NOS-detect assay kit.

2.9 Estimation of plasma TNF-α level

Blood samples in EDTA- containing vials were centrifuged at 1000 rpm for 12 min at 4°C. The levels of TNF-alpha were determined by using an ELISA kit.

2.10 Statistical Analysis

All values are reported as the mean ± S.E.M. and were analyzed by one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons using the Statistical Package for the Social Sciences software (SPSS 20). The differences between means were considered statistically significant when the 'p' value was less than 0.05.

3. Results

The results of this study are depicted in Table 1 & 2 as well as in Fig.1-3. The results display the free acidity levels and the estimation of the levels of PGE2, iNOS, TNF alpha and H, K ATPase enzyme. The results suggests that most anti-ulcer agents like the PUFA containing oils reduce H, KATPase, TNF alpha, iNOS while increasing the PGE2 levels. Free acidity levels were also substantially reduced by the treatment with PUFA containing oils and conventional anti-ulcer drugs like Omeprazole and Ranitidine.

Figure 1: PGE_2 levels after drug treatment

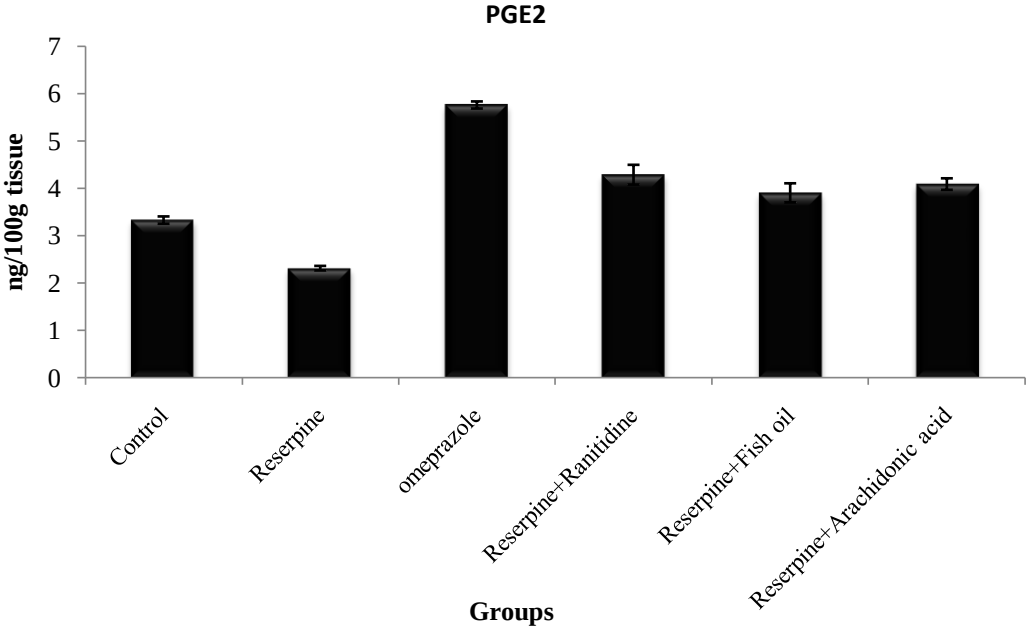


Figure 2: Inducible nitric oxide synthase levels in ulcerated gastric mucosa after drug treatment

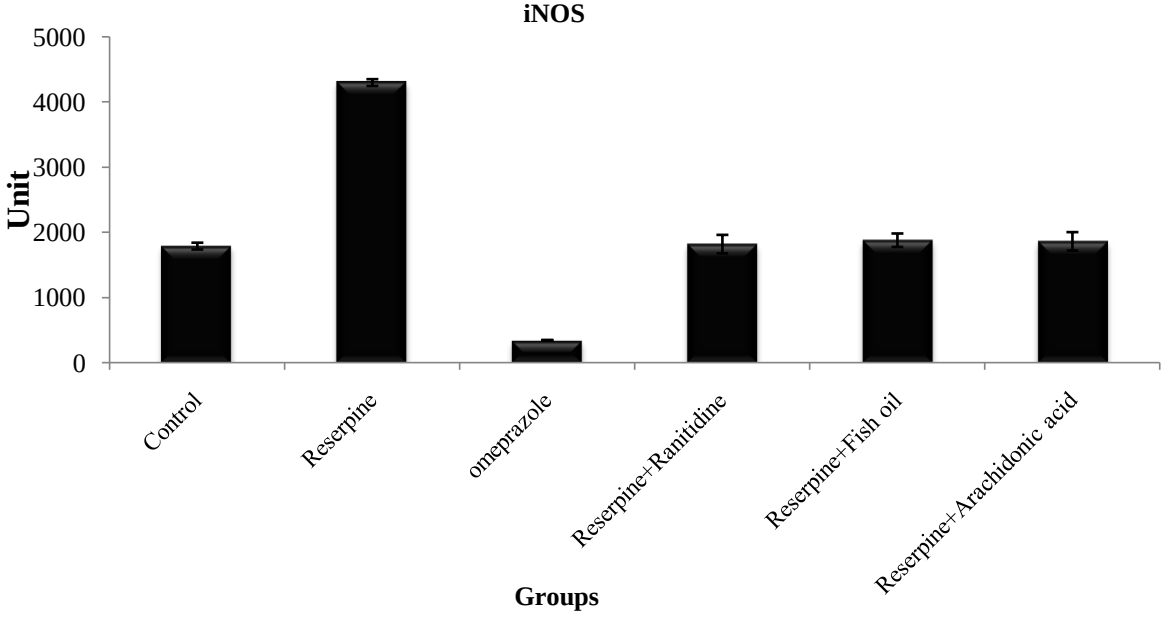


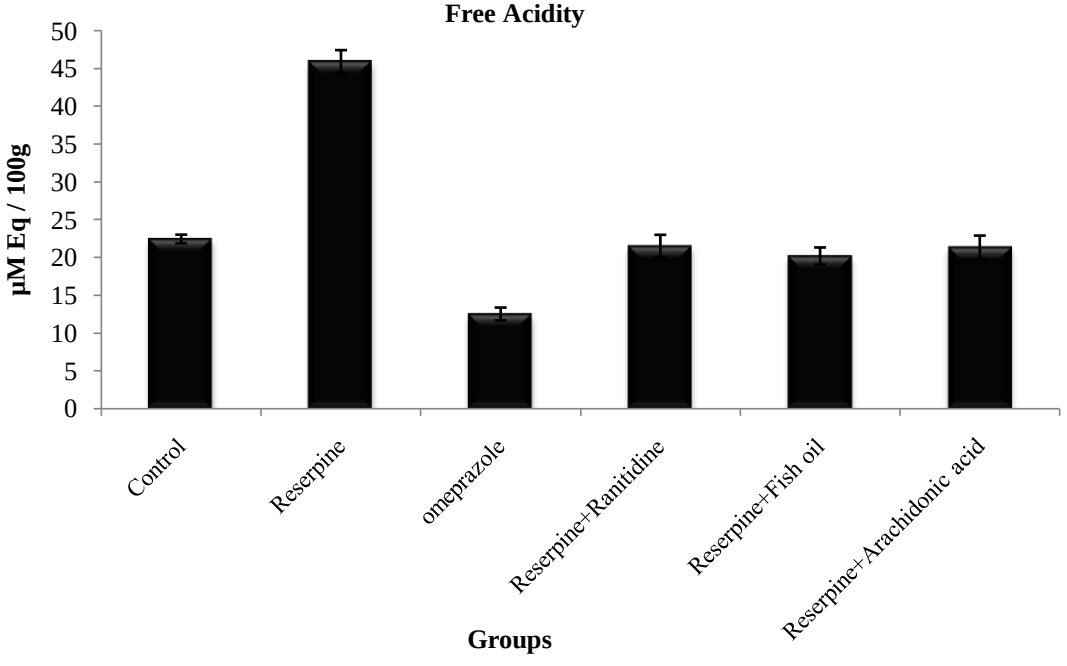
Table 1: TNF-alpha levels after treatment with various drugs

Group No.	Treatment (mg/kg)	TNF-alpha Mean ± SEM
Gp -I	Control	63.6 ±1.99
Gp-II	Ulcerated control(Reserpine 10mg/kg,p.o)	98.6 ±4.69
Gp-III	Omeprazole 20mg/kg	29.8 ±2.19
Gp-IV	Ranitidine (30 mg/kg.p.o)prior to reserpine	68.8 ±5.57
Gp-V	Fish oil(40µl/day.p.o) followed by reserpine	70.8 ±5.79
Gp-VI	Arachidonic acid40µl/day.p.o) followed by reserpine	58.8 ±2.77

Table 2: Inhibition of H, K-ATPase activity in reserpine induced ulcer models

Group No.	Treatment (mg/kg)	H,K-ATPase Mean ± SEM
Gp -I	Control	1.27±.073
Gp-II	Ulcerated control(Reserpine 10mg/kg,p.o)	3.20±.16
Gp-III	Omeprazole 20mg/kg	0.93±.049
Gp-IV	Ranitidine (30mg/kg.p.o)prior to reserpine	1.54±.071
Gp-V	Fish oil(40µl/day.p.o) followed by reserpine	1.31±.034
Gp-VI	Arachidonic acid40µl/day.p.o) followed by reserpine	1.30±.065

Figure 3: Free acidity levels after drug treatment



4. Discussion

The results of this study are shown in Table 1 & 2 and Figures 1-3. Yamaguchi *et al* reported that reserpine increased acid secretion in rats[12], which was a possible mechanism leading to mucosal lesions. However, the exact mechanism is not yet clear. Reserpine was believed to exhaust the monoamines at the ends of sympathetic nerves, leading to over activity of the vagal nervous system at the peripheral level. This may lead to an over secretion of gastric acid. In humans, apart from *H.Pylori* and NSAIDs and alcohol it is stress which can arise from prolonged anxiety, tension, and emotion, severe physical discomfort, haemorrhage and surgical shock, burns and trauma, thereby resulting in severe gastric/peptic ulceration. The gastric acid is an important contributor for the genesis of ulceration in experimentally induced animal models. Current gastric ulcer therapies show moderate efficacy against gastric mucosal lesions/ulceration but are also associated with several side effects and there is always look out for more nutraceuticals based therapy for gastric ulcer disorders like the gastric/peptic ulcers. Hence the studies are being conducted on natural products which can either settle peptic ulceration or reduce hyperacidity to a normal level so that stomach can functions its physiological role. PUFA are such agents that can come to some expectations in this regard. They may or may not be as good as modern medicines used in hyperacidity or peptic ulceration, however they can allow stomach to function normally which can serve the purpose and balance can be established in this regard. The secretion of gastric acid can increase the incidence of peptic ulcer disease. Maintaining

adequate acid secretion at a normal level is the main therapeutic target of any antacid therapy. Currently the experimental *in vivo* models still are the best and cost effective way to evaluate the efficacy and potency of novel anti-ulcer drugs and their mechanisms. One of the most enzyme mediating acid formation is the H,KATPase. The H,K-ATPase is the dimeric enzyme responsible for H^+ secretion by the gastric parietal cells. H,K-ATPase is selectively blockedby the action of Lansoprazole, an acid blocker used for treating gastric ulcers. Activation of cAMP pathway stimulates the H,K-ATPase on parietal cells, a high capacity proton pump, with its insertion into the apical membrane leads to the formation of a secretory canaliculi. In the recent years, the drugs that reduce the acid secretion and H,K-ATPase inhibition have become preferred therapeutic choice due to their clinical efficacy. The inhibition of H,K-ATPase results in the reduction of gastric acid secretion which is concordant with the results of the present study.

In this study, we used reserpine induced model of ulcer formation. Reserpine is an anti-hypertensive drug which causes catecholamine depletion. Reserpine was believed to exhaust the monoamines at the ends of sympathetic nerves, leading to over activity of the vagal nervous system at the peripheral level. This may lead to an oversecretion of gastric acid. Reserpine is also documented to generate free redicals and inhibit the prostaglandin synthesis [13][14]. It has appeared that peripheral cholinergic and adrenergic mechanisms are involved in the ulceration induced by reserpine [15].

The data presented here provided scientific evidence that production of TNF- increases the risk of gastric ulcer. Suppression antisecretory activity, as observed by the decrease in of TNF- and IL-1 production this may be attributed to total acidity and volume of gastric juice [16]. Further, the the anti-inflammatory activity of these n3 and n6 containing PUFA containing oils reduced the TNF alpha levels and increased the PGE_2 levels[17]. This treatment offers cytoprotection by increasing inhibition of TNF-α and neutrophil infiltration in mucus. Thus these PUFA containing oils ultimately inhibit tissue destruction by reactive oxygen showed good gastro protective anti-ulcerogenic activity species. Prostaglandin, possess antiulcer activity against the ulceration and an important agent as its inhibition is responsible for complex array of ulcer by aspirin[18]. Aspirin a well known NSAID inhibits PGE2 which has healing mechanism, blocks the synthesis of gastroprotective prostaglandins which are synthesized in the mucosal cells erosions and ulcers in gastroduodenal tract by cyclooxygenase (COX) enzyme action. Prostaglandins are found to be inhibiting the leukocyte recruitment which could contribute to the beneficial effects of these substances in situations in which the GI mucosa is inflamed [19].

On the other hand, the free radical NO is deleterious for the gastric mucosa. Suppression of NO synthesis renders the gastric mucosa more susceptible to injury. NO inhibits recruitment of neutrophils to sites of inflammation. NO reduces neutrophil infiltration into the GI tract mucosa [20][21]. The events related to the gastroprotective effects of nitric oxide include a reduction in acid secretion and promotion of angiogenesis. However the iNOS enzyme has different roles to play as well and the inducible NOS which produces relatively large of NO under certain pathological conditions, contribute to mucosal injury and dysfunction[22][23]. Several plant products contain flavonoids which have inhibitory action on iNOS enzyme [24].

The data obtained from our study suggests that the PUFA containing oils used in this study i.e. the fish oil and Arasco oil the n-6 PUFA containing Arasco oil were able to attenuate the ulcer formation as calculated based on the ulcer index[25]. Dietary supplementation with n-6 fatty acid rich in LA has been found to influence the physiological function of various blood components, producing an inhibitory effect on leucocyte adhesion, platelet count, platelet aggregation and collagen formation [26]. Dietary supplementation with n-3 PUFAs improved colonic anastomoses healing. n-3 PUFAs enhance the colonic wound healing in a rat model. Actually, n-3 PUFAs may prompt faster resolution of inflammation within the wound microenvironment, which leads to facilitated regeneration and re-epithelialization[27]. A small randomized controlled trial evaluated a formula supplemented with fish oil in patients with pressure ulcers and noted decreased progression of pressure ulcers in those receiving fish oil supplementation. There is growing evidence that the diverse biological roles of n-3 PUFAs contribute to their regenerative actions against chronic inflammatory disease. This could effectively help resolve the inflammation and promote a transition from the inflammatory to the proliferative and remodeling phases of wound healing. Biochemical events are the factors possibly contributing to the processes underlying ulcerogenesis, in the present experimental models. It is therefore speculated that the anti-secretory activity of PUFA may account for antiulcer activity in various experimental models used in the present study, where gastric secretion is involved in the pathogenesis of gastric ulcers. These results have shown that PUFA containing oils provided moderate gastrointestinal protection in all the induced ulcer models. Thus it can be concluded that PUFA containing oils like the Fish oil and Arasco oil have antiulcer properties.

5. Conclusion

Our present study suggests that PUFA containing oils possess antioxidant, inflammatory actions and reduce H, KATPase, iNOS and TNF alpha levels. The effects of PUFA containing oils and the antacid drugs have suppressive actions on inflammatory mediators of the gastric mucosa in the reserpine induce gastric ulcer rat model. A strategy involving

either the combination of nutraceuticals oils and antacid agents can be useful for treating cases of chronic ulcer patients. There is also need to elucidate the active principle present in these oils which may be aiding in suppression of gastric ulcer formation.

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