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EFFECT OF OILS WITH PUFA AND ANTI-ULCER DRUGS ON IL-1 BETA AND TNF-ALPHA LEVELS IN DIMAPRIT INDUCED ULCERS

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ABSTRACT
Peptic ulcers are a mucosal lesion of the stomach or duodenum in which the acid and pepsin play major pathogenic roles. The major forms of peptic ulcer are gastric ulcer and duodenal ulcer, both of which are chronic diseases often caused by the bacteria, Helicobacter pylori. The term peptic ulcer also encompasses gastric ulcers and duodenal ulcers associated with stress or the ingestion of drugs, most commonly non-steroidal anti-inflammatory drugs (NSAIDs) and alcohol. Ulcer associated with Zollinger-Ellison Syndrome (ZES), caused by gastrin secreting G cells is also considered a form of peptic ulcer. Biochemical mediators like the interleukins and TNF have been implicated in the genesis of peptic ulcer. This article evaluates the effects of PUFA containing oils and anti-ulcer drugs on interleukin-1 beta and TNF alpha levels in dimaprit induced ulcers.

KEYWORDS: Histamine, dimaprit, TNF-alpha, Ulcer, Omeprazole.

INTRODUCTION
There are several models that are used to evaluate antiulcer medicines. However, the choice of a suitable model has proven to be difficult as each model has significant advantages as well as disadvantages. The choice of a particular model is sometimes influenced by local resources, the objectives of the study, the hypothesis being tested, or research questions being answered by the researcher. Histamine is one of the important aminergic neurotransmitters and plays a significant role in the regulation of several patho-physiological processes including gastrointestinal ulcers.[1,2] Histamine is found in every human tissue and can act as a local hormone, a mediator in processes related to allergy and inflammation, or a neurotransmitter.[3] Histamine exerts its effect through H_1,H_2,H_3 and H_4 receptors.[4] In particular, the H_2 histamine receptor (H_2R) is coupled to the Gs protein and adenylyl cyclase system in a variety of tissues e.g., brain, stomach, heart, gastric mucosa, lung and produces intracellular cAMP.[5] Gantz et al.[6] were the first to clone a cDNA encoding a 359-amino acid H2R. Using degenerate primers based on the known sequence similarity of various G protein-coupled receptors, the H2R sequence was obtained from canine gastric parietal cDNA by using the PCR technique. Soon thereafter, the intronless genes encoding the rat, human, guinea pig and mouse H_2R were cloned by means of homology screening.[7] Many reports demonstrated the cellular function of H_2R and its importance. In immune system, the responses of T helper cells (Th1 and Th2) are negatively regulated by H_2R through the activation of different biochemical intracellular signals. Histamine does not only enhance gastric acid secretion, but it also causes disturbances of the gastric mucosa, microcirculation, abnormal motility and reduction in mucus production. The mechanism by which histamine induces gastric ulcers is through its potent acid stimulating and vasodilating capability, which leads to increased vascular permeability. Dimparit is a histamine agonist and is used to evoke gastric erosion and induce experimental form of ulcers in animals. This study was conducted to evaluate the effects oils with PUFA and anti-ulcer drugs on dimaprit induced ulcers in rats and effects on biochemical mediators like the interleukin-1 beta and TNF alpha.

MATERIALS AND METHODS
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.

Drugs and chemicals

Fish oil - an n3 rich oil Maxepa (EPA & DHA) was procured from Merck, India and Arachidonic acid – rich oil 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 administered orally.

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. Dimaprit was injected intravenously.

Group- I: Rats received only 0.1% Tween 80, served as vehicle control.
Group- II: Rats received dimaprit is given in a dose of 150mg/kg IV, single dose administered, treated as ulcerated control,
Group- III: Rats received omeprazole (20 mg/ kg) served as positive control,
Group- IV: Rats received Ranitidine (30 mg/kg, p.o.+ dimaprit.
Group- V: Rats received fish oil 40µl/day/animal for 10 days + dimaprit.
Group- VI: Rats received AA-rich oil 40µl/day/animal 10 days+ dimaprit.

Determination of free acidity

The free acidity was calculated as per previously shown formula.[8] 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). The volume of alkali added was noted. This volume corresponds to free acidity. Later 2-3 drops of phenolphthalein solution were added and titration was continued until a defined red tinge reappears. 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.

Estimation of plasma IL-beta and TNF-α levels

Blood samples in EDTA- containing vials were centrifuged at 1200 rpm for 10 min at 4°C. For IL-1 beta the Intra-assay coefficient of variation and sensitivity of the method were 4.8% and 0.3 nM/100g tissue. The levels of TNF-alpha and IL-beta were determined by using an ELISA kit.[9]

Dimaprit induced duodenal ulcer

Dimaprit, an H_2 receptor agonist, has been shown to induce gastric erosions in rats after a single iv/ip dose. This model is especially useful for screening of H_2 blockers.

Procedure

Wistar rats (250 to 300g) are used for the experiment. The animals are fasted for 24 hours before the experiment but allowed free access to water. In rats, dimaprit is given in a dose of 150mg/kg iv, single dose. The animal is sacrificed one hour later and the stomach dissected out and examined for gastric erosions. The test drug or vehicle is given orally 60 minutes before injecting dimaprit.

RESULTS

The results of our study are described in Table 1 and Figures 1 & 2. The data described are about the free acidity levels and levels of TNF alpha and IL-1 beta after induction of ulcers by Dimaprit. It is evident from the results that the levels of free acidity are reduced substantially by anti ulcer drugs and PUFA containing oils like the fish oil and Arasco oil. A similar trend of attenuation of levels in TNF alpha and IL-1 beta are seen after anti ulcer drugs like Omeprazole and Ranitidine as well as the PUFA containing oils.

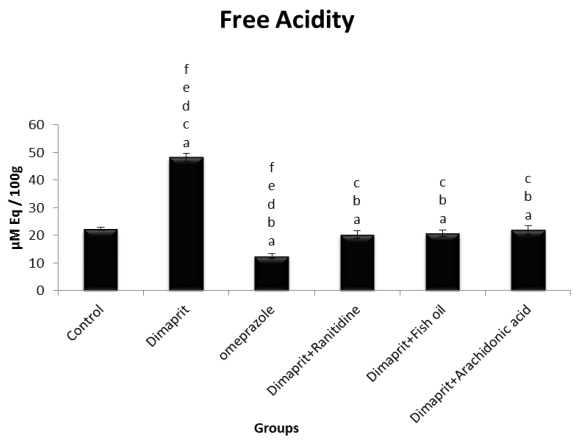

Fig. 1.

Each represents the mean ± SEM of data from six animals. The statistical significance of the data is $p<0.05$. a-Control *vs.* others; b-Dimaprit *vs.* others; c-Omeprazole vs others, d-Ranitidine+ Dimaprit vs others; e-Fish oil+ Dimaprit vs others; f- AA+ Dimaprit vs others.

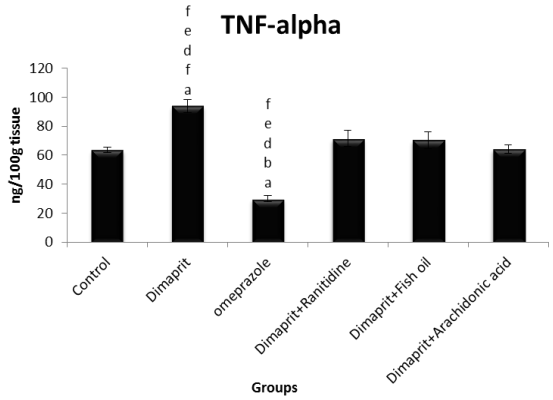

Fig. 2.

Each represents the mean ± SEM of data from six animals. The statistical significance of the data is p<0.05. a-Control *vs.* others; b-Dimaprit *vs*. others; c-Omeprazole vs others, d-Ranitidine+ Dimaprit vs others; e-Fish oil+ Dimaprit vs others; f- AA+ Dimaprit vs others.

Table 1: Effect of fish oil and Arasco oil in comparison with standard drugs on IL1-beta in Dimaprit induced gastric ulcer in rats.

Group No.	Treatment (mg/kg)	IL1beta levels (nM/100g tissue)
Gp -I	Control	104.167± 6.73
Gp-II	Ulcerated control(Dimaprit 150mg/kg,i.p)	193±3.59
Gp-III	Omeprazole 20mg/kg	71±3.69*
Gp-IV	Ranitidine (30mg/kg.p.o)prior to Dimaprit	128±6.042*
Gp-V	Fish oil(40µl/day.p.o) followed by Dimaprit	159±8.35*
Gp-VI	Arachidonic acid40µl/day.p.o) followed by Dimaprit	138±5.029*

Each represents the mean ± SEM of data from six animals. The statistical significance of the data is p<0.05. *= Dimaprit treated control *vs*. drug treated groups.

DISCUSSION

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.[10,11] The gastric acid is also 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 perform its physiological role.[12] In recent years, there has been a growing interest in alternative therapies, especially those from plants and other natural products due to their perceived relative lower side effects, ease of accessibility and affordability. Plant medicines with ethnomedicinal use in peptic ulcer management need to be screened for their effectiveness and possible isolation of lead compounds.[13] This requires use of appropriate animal models of various ulcers. The limited number of antiulcer models for drug development against gastric and duodenal ulcer studies has hindered the progress of targeted therapy in this field and PUFA are such agents that can come to some expectations in this regard. PUFAs are fatty acids some of which have at least two carbon-to-carbon double bonds in a hydrophobic hydrocarbon chain, which typically includes X-Y carbon atoms and terminates in a carboxylic acid group.[14,15] On the other hand *in vitro* studies demonstrate that H2R agonists mimic the actions of histamine, which inhibits the secretion of proinflammatory cytokines and stimulates the production of anti-inflammatory cytokines in human peripheral blood mononuclear cells. Furthermore, the effects induced by histamine were primarily mediated by H_2R, evidenced by the fact that these effects were blocked by cimetidine. In addition, the H_2R mediates suppression of TNF-alpha production by mast cells.[16] IL-1beta, is also relevant to the pathogenesis of peptic ulcer disease.[17] In an interesting publication, Watanabe and colleagues attempt to define the role of IL-1 and gastric acid secretion in gastric ulcer recurrence. They used an established rat model in which antral ulcers, induced by submucosal injection of 20% acetic acid, are known to recur on intra-peritoneal injection of IL-1. They found that 24 hours following IL-1 injection, expression of adhesion molecules and concentrations of IL-1beta and TNF-alpha in scar tissue had increased.[18]

Our data are consistent with previous studies showing that oils with PUFA inhibits histamine induced TNF-α production from monocytes and mast cells.[19] The data shown in Table 1 and Fig. 1 & 2 clearly shows the anti-acid properties of oils with PUFA as well as their prominent action on IL-1 beta and TNF-alpha levels. The data presented here provided scientific evidence that production of TNF- increases the risk of gastric ulcer.

Suppression of 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.[20] Further, the the anti-inflammatory activity of these n3 and n6 containing PUFA containing oils reduced the TNF alpha levels. This treatment offers cytoprotection by increasing inhibition of TNF-α and neutrophil infiltration in mucus. There are at least two independent families of PUFAs, depending on the parent fatty acid from which they are synthesized. They include: the "ω-3" series derived from ALA (18:3, ω-3); the "ω-6" series derived from *cis*-LA (18:2, ω-6).[21] Thus these PUFA containing oils ultimately inhibit tissue destruction by reactive oxygen showed good gastro protective anti-ulcerogenic activity species. 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.[22] 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.[23] 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 partially for antiulcer activity in various experimental models including one used in the present study, where gastric secretion is involved in the pathogenesis of gastric ulcers. There is need to conduct more comprehensive pre-clinical studies with these oils with PUFA to elucidate their molecular mechanisms in anti-ulcer activity.

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