COMPARATIVE EFFECT OF FLAXSEED OIL AND FISH OIL IN ACETIC ACID- INDUCED COLITIS IN RATS

Aisha Dugani¹, Ahlam Elhelawi¹, Aisha Edrah², ¹⁾ Department of Pharmacology and Clinical Pharmacy, University of Tripoli, Tripoli, Libya, ²⁾ Department of Pharmacology, Faculty of Medicine, Tripoli University, Tripoli, Libya.

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Corresponding author: Aisha Dugani, e- mail: duganiaisha@yahoo.com

ABSTRACT:

Objectives: The aim of the present study was to evaluate the possible protective effect of flaxseed oil and to compare this effect with fish oil in experimental ulcerative colitis (UC).

Methods: Rats were equally divided into five groups of six animals each. Sham control group (corn oil, 1 ml), acetic acid group (normal saline, 1 ml), flaxseed oil group (FSO, 1 ml), fish oil group (FO, 1 ml) and mesalamine-treated group (3 ml) as a positive control. All drugs were administered intrarectally (IR). One hour following treatment in the acetic acid group, FSO group, FO group and mesalamine group, 1 ml of 4% acetic acid was introduced as an enema. Rats were sacrificed after 24 hrs and histopathological scores of the all colonic specimens were assessed by microscope. Colonic weight/length ratio was also evaluated.

Results: Microscopical improvement as manifested by the reduction in the inflammatory score and normalization of intestinal mucosal architecture was observed in fish oil pretreated rats compared to acetic acid group but there was no significant difference in flaxseed oil pretreated group. The decrease in weight/length ratio was statistically significant in fish oil-treated group compared with acetic acid group, but there was no significant difference between flaxseed oil-treated and acetic acid group.

Conclusion: The results of this study suggest that fish oil but not flaxseed oil could ameliorate the mucosal damage in experimentally induced ulcerative colitis in rats when given in the form of an enema.

Keywords: Flaxseed oil; ulcerative colitis; fish oil; acetic acid induced colitis.

الملخص العربي:

دراسة لمقارنة تأثير زيت بذرة الكتان بتأثير زيت السمك على التهابات الناشئة عن حمض الخل في

القولون عند الفئران

عائشة الدوجاني^{1*}، أحلام الهلاوي¹، وعائشة دراه²

قسم علم الأدوية والصيدلة السريرية، كلية الصيدلة، 2 قسم علم الأدوية، كلية الطب، جامعة طرابلس، طرابلس، ليبيا.

الهدف: تهدف هذه الدراسة إلى تقييم التأثير لزيت بذرة الكتان ومقارنته بتأثير زيت السمك في الوقاية من التهابات القولون عند الفئران. **الطرق:** قسمت الفئران إلى خمس مجموعات متساوية، بكل منها ستة فئران. المجموعة الأولى، تعتبر المجموعة الضابطة، أعطيت زيت الذرة (1 ملي لتر). المجموعة الثانية، مجموعة حمض الخل، فأعطيت محلولاً ملحي (1 ملي لتر). المجموعة الثالثة، مجموعة زيت السمك، أعطيت زيت السمك (1 ملي لتر). المجموعة الرابعة، مجموعة زيت بذرة الكتان، فأعطيت واحد ملي لتر من زيت بذرة الكتان. أما المجموعة الخامسة، المجموعة الضابطة الموجبة، فأعطيت 3 ملي لتر من عقار الميزالامين mesalamine. كل الجرعات تم إعطاؤها من طريق الشرج. وبعد ساعة تم إعطاء جميع المجاميع ما عدي المجموعة الأولى واحد ملي لتر من 4% حمض الخل من طريق الشرج. وبعد 24 ساعة تم قتل جميع الحيوانات، وأخذت عينات من القولون لقياس طولها وتعيين وزنها وحساب النسبة بين طول القطعة إلى وزنها، وتم أيضاً التقييم المجهرى للتغيرات النسيجية المرضية على نسيج القولون لجميع العينات.

النتائج: بينت هذه الدراسة أن إعطاء زيت السمك من طريق الشرج قبل استحداث التهاب القولون التقرحي بواسطة حمض الخل، أدى إلى انخفاض في معدل التهاب القولون، وتحسن في الحالة النسيجية المرضية للقولون، في حين لم يكن للمعالجة الشرجية موضعية لزيت بذرة الكتان أي تأثير على التهابات القولون.

الاستنتاجات: بينت نتائج هذه الدراسة أن حقن زيت السمك، وليس زيت بذرة الكتان، من طريق الشرج له تأثير جيد في التقليل من التخرشات التي يحدثها حمض الخل في جدار القولون عند الفئران.

الكلمات مفتاحية: زيت بذرة الكتان، زيت السمك، التهاب القولون التقرحي، حمض الخل.

INTRODUCTION:

Ulcerative colitis (UC) is a form of inflammatory bowel disease (IBD) characterized by diffuse mucosal inflammation of the colon and rectum and typically involves only the innermost lining of the mucosa, manifesting as continuous areas of inflammation and ulceration with no segments of normal tissue [1]. The disease typically starts in the rectum, but often extends to involve the whole length of the colon [2]. Despite the enormous research on the pathogenesis of UC, the exact cause of the condition remains not completely understood. The disease appears to be related to combination of genetic and environmental factors [1]. The pathological findings associated with UC include: an increase in inflammatory mediators such as prostaglandins (PG) and leukotrienes (LT), which are produced from arachidonic acid. Clinical and experimental studies have confirmed that the levels of prostaglandin E₂, thromboxane A₂, prostacyclin, and especially LTB₄ during colonic inflammation were highly increased [3]. Other factors include: oxidative stress, altered colonic milieu, abnormal mucosal content of glycosaminoglycan (GAG), decreased oxidation of short chain fatty acids (SCFAs), increased intestinal permeability, increased sulfide production, and decreased methylation [1]. Treatment of IBD including UC consists of sulphasalazine (SSZ), 5-aminosalicylic acid (5-ASA), corticosteroids, immunomodulator drugs [azathioprine (AZA) and methotrexate (MTX)], 6 mercaptopurine (6-MP), calcineurin inhibitors (cyclosporin and tacrolimus), and anti-TNF- α antibodies (infliximab, adalimumab and certolizumab). The choice of treatment depends on the clinical goal (induction or maintenance of remission), extent and severity of disease, response to current or prior medication and the presence of complications [4]. Polyunsaturated fatty acids (FA) of the omega-3 class, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have attracted interest in recent years because of their anti-inflammatory properties [5]. Several published research work have confirmed the protective effect of fish oil supplemented in diet or as enema in experimental induced colitis [5-8] and also in clinical trials on patients with IBD [9-13]. Thus it was thought worthwhile to investigate the effect of other omega-3 rich oil such as flaxseed oil and to study the comparative effects of these two oils in the protection against acetic acid inducing colitis in rats.

MATERIALS AND METHODS: ANIMALS.

Healthy Albino Wistar rats of either sex weighing 200-250 g were used in this study. They were randomly allocated to groups of 6 rats each. The animals were housed in the animal care facility in the department of Pharmacology and Clinical Pharmacy and maintained at 23°C with a 12 hour light and 12 hour dark cycle. All rats were fasted for 24 hours prior to the experimental procedure. The study was approved by the Faculty of Pharmacy and the experiments were done according to the ethics guidelines of Tripoli University.

INDUCTION OF COLITIS AND TREATMENT PROTOCOL:

Colitis was induced in rats by intrarectal (IR) administration of acetic acid (AA). After anesthetized with ketamine in a dose of 75 mg/kg injected intraperitoneally, a soft 6F polypropylene catheter lubricated with jelly was inserted 6-8 cm via the anal canal into the colon. One ml of AA (4% vol./vol. in 0.9% saline) was slowly infused into the distal colon and rats were maintained in a head-down position for 30 seconds to limit the expulsion of the solution [5].

TREATMENT PROTOCOLS:

Rats were randomized into five groups. Group 1: Sham control group (SC). One ml of normal saline was given IR after 1 hour from administration of one ml corn oil enema. Group 2: Control colitis group (CC), one ml of 4% AA was administered IR 1 hour following IR administration of normal saline. Group 3: Fish oil treatment group (FO-IR), one ml of FO was administered as enema one hour before induction of colitis. Group 4: Flaxseed oil treatment group (FSO-IR), one ml of FSO was administered as enema one hour before induction of colitis. Group 5: mesalazine treatment group, the animals were given 3 ml of mesalazine enema one hour before induction of colitis. One hour following the above treatments, colitis was induced in all animal in groups 3, 4 and 5 by the administration of AA IR (1.0 ml of 4%). All rats were scarified 24 hours following AA-treatment.

ASSESSMENT OF COLONIC DAMAGE:

After midline laparotomy, the colon was removed, cleaned from fat and mesentery, and blotted on a filter paper. Each specimen was weighed and its length measured under a constant weight (2 g) and expressed as colon weight (mg)/length (cm) ratio. The distal 2-3cm segment of the colon was isolated from the rest of the tissue samples and opened longitudinally, rinsed with saline to remove luminal content and fixed in 10% formalin and embedded in paraffin, and 5µm sections were prepared. Tissues were routinely stained with hematoxylin and eosin and were evaluated for the appearance of muscle layers by a light microscopy by a pathologist who was not informed about the treatment protocols. The degree of colonic inflammation was evaluated by histologic scoring (from 0 to 3) as previously describe [14], where normal histologic appearance = 0; 1= inflammation on mucosa and submucosa; 2= inflammation on entire wall of the bowel, and 3 = ulcer and necrosis of the entire wall.

STATISTICAL ANALYSIS:

Data are expressed as mean $\pm$ S.E.M. Statistical calculations were done with SPSS 11.0 software package. Comparison between two groups was performed using Student's t-test and comparison between more than two groups was carried using one-way analysis of variance (ANOVA). Differences were considered significant when the degree of confidence was 95% or better ($P < 0.05$).

RESULTS:

The intracolonic administration of 1.0 ml of 4% AA produced a severe and significant inflammatory response in rat colon 24 hrs after injection. This response is evidenced by the significant increase in weight of 5 cm distal colonic segment and an increase in the histopathological score as compared to control group treated with normal saline (Figure 1 and Figure 2B). All rats in the acetic acid treatment group developed diarrhea, in some animals bloody diarrhea was observed.

Figure1. Effect of pretreatment with fish oil, flaxseed oil and mesalamine on colon weight/length ratio in acetic acid- induced colitis in rats. Values are mean $\pm$ SEM, n = 6. #, $P < 0.001$ significantly higher than sham control (normal saline-treated); *, $P < 0.05$ and **, $P < 0.01$ significantly lower from colitis group (acetic acid-treated group) respectively.

EFFECTS OF FISH OIL AND FLAXSEED OIL ON COLONIC WEIGHT/ LENGTH RATIO IN ACETIC ACID- INDUCED COLITIS.

The colonic tissue segment weight/length ratio was significantly higher in the AA-group as compared to SC group (37.0 ± 1.0 vs 23.12 ± 0.9 respectively, $P < 0.001$, Figure 1. Pretreatment of rats with one ml of FO-IR, showed a significant mucosal protection effect against AA-induced colitis as evidenced by the decrease in the colon weight/length ratio in comparison with SC-treatment groups (32.97 ± 1.02 vs 37.00 ± 1.01 , $P < 0.05$). FSO pretreatment, on the other hand, has a slight but insignificant reduction in the colonic tissue weight/length ratio in comparison with AA- control group (36.00 ± 1.9 vs 37.00 ± 1.01 respectively Figure 1.

EFFECT OF FISH OIL AND FLAXSEED OIL ON COLONIC HISTOLOGICAL DAMAGE INDUCED BY ACETIC ACID.

Representative histological segments of the colon in the control, AA- treatment group and in group of rats treated with FO, FSO and mesalamine enemas are shown in figure 2. AA produced a significant inflammatory response and prominent lesions in the mucosa and submucosa, with invasion of inflammatory cells mainly macrophages and neutrophils and dilation of capillaries and lymphatics, Figures 2B and 3.

Figure 2. Examples of photomicrographs of rat colonic mucosa. (A) Sham negative control rat (saline-treated), (B) Acetic acid-treated rat, (C) Fish oil-treated rat, (D) Flaxseed oil-treated rats, (E) Mesalamine treated group. Arrows and circle indicate ulcerated epithelium.

EFFECT OF FISH OIL AND FLAXSEED OIL ON COLONIC HISTOLOGICAL DAMAGE INDUCED BY ACETIC ACID

Representative histological segments of the colon in the control, AA- treatment group and in group of rats treated with FO, FSO and mesalamine enemas are shown in figure 2. AA produced a significant inflammatory response and prominent lesions in the mucosa and submucosa, with invasion of inflammatory cells mainly macrophages and neutrophils and dilation of capillaries and lymphatics, Figures 2B and 3. FO treatment resulted in correction of the morphological disturbances associated with AA administration. The histopathological score was significantly reduced from 3.7 ± 0.2 in the AA colitis group to 1.2 ± 0.4 in the FO-treatment group respectively, ($P < 0.01$) (Figure 2 C). FSO, on the other hand, has no effect on tissue damage caused by AA, the histological damage score in this group was 3.2 ± 0.5 versus 3.7 ± 0.2 in AA control group and the reduction was not significant. Comparing the results of FO group with the standard drug mesalamine, the reduction in histological score was slight and insignificant (1.2 ± 0.4 vs 1.5 ± 0.6). The findings at microscopy were consistent with macroscopic appearance and changes in colonic weight and the weight/length ratio in the different treatment groups. These results are summarized in, figure 4.

Figure 3. Higher magnification of rat colon treated with acetic acid showing in A, B and C: large number of mucosal glands with hyperplastic (1) and dysplastic (2) changes with some of them lined by one or more layers and others seen completely obstructed with cystic formation (3) or papillary cystic formation. The submucosal blood vessels are severely dilated and hyperemic (4)

Figure 4. Effect of pretreatment with fish oil, flaxseed oil and mesalamine on histological damage score in acetic acid-induced colitis model in rats. Values are mean $\pm$ SEM, $n = 6$. \$, $P < 0.01$ significantly higher than normal saline (negative control) group. #, $P < 0.05$ significantly higher than fish oil; **, $P < 0.01$ significantly lower than acetic acid (control colitis group).

DISCUSSION:

The present study was planned to compare the protective effects of two omega-3 rich oils; the plant derived FSO and the marine derived FO on AA colitis in rats. Induction of colitis by AA is one of the standard methods to produce a model with the characteristics of human ulcerative colitis [15]. It affects the distal colon portion and induces non-transmural inflammation, massive necrosis of mucosal and submucosal layers, mucosal edema, neutrophil infiltration of mucosal and submucosal ulceration [16]. The inflammatory response initiated by AA includes activation of cyclooxygenase and lipoxygenase pathways [17]. The reason behind investigating the protective effects of FSO in this study and to compare its effects with FO, is the fact that FSO by far is the richest source of Omega-3 fatty acid alpha-linolenic acid (ALA) at roughly 57%, six times richer than most fish oil, in n-3 [18,19]. Its

omega6:omega3 ratio is very desirable at 0.3:1. The ALA is converted by metabolism in vivo into the longer chain omega 3's EPA and DHA. To our knowledge, there have been no reports on the effects of the plant derived FSO either as supplement or in the form of enema in experimental colitis. Our results have demonstrated that intracolonic administration of 4% AA caused a substantial degree of inflammation and tissue injury in the rat colon as evidenced microscopically by the increase in cellular infiltration, muscle thickness and loss of architectural structure and macroscopically by the increase in weight of colon segment compared with SC-group. The degree of tissue damage was markedly attenuated by intrarectal treatment with FO. These results are in accordance with other studies, which demonstrated that FO enema provided a protective effect against ethanol, AA and trinitrobenzene-induced colitis models in rats [5, 6]. The protective effect was reported to be associated with a significant reduction in the levels of leukotriene B4 and myeloperoxidase activity in the colonic tissue [5]. On the other hand, we were unable to detect any protective effect of FSO in AA-induced colitis. Clinical reports [9-13] have indicated that in patients with UC, supplementation with FO was effective in reducing both the symptoms and the dose of corticosteroid required to control the disease and in reducing rate of relapse. In a case report study, FO enema produced a significant improvement in symptoms, normal-appearing colonic mucosa on colonoscopy and on histopathology [20]. The exact mechanism of omega-3 fatty acids (n-3 FA fatty acids) involved in the attenuation of inflammations is not well understood. However, they n-3 FA are believed to reduce inflammation through reduction of the incorporation of EPA and DHA into the arachidonic acid with increased production of 3-series PGs and thromboxanes and 5-series LTs, thus reduce the inflammatory potential [21, 22]. Interleukin-1 α and tumor necrosis factor production may also be reduced by the presence of n-3 FA fatty acids [23]. Thus, the propagation of inflammatory pathways is diminished. The n-3 FA fatty acids from FO have been found to inhibit cytokine and eicosanoid formation. Prolonged treatment with FO in patients with rheumatoid arthritis (RA), resulted in a significant decrease in LTB4 and TNF- α [24]. Eicosapentaenoic acid EPA is the precursor of the PG3 series of prostaglandins and resolvins, which have anti-inflammatory effects. Docosahexaenoic acid DHA is the precursor of the docosanoids, termed 'resolvins' or 'protectins', which are analogous to the eicosanoids and have

potent anti-inflammatory and immune-regulatory actions [25, 26]. Docosahexaenoic acid DHA is believed to have beneficial effects upon inflammatory disorders of the intestine and in reducing the risk of colon cancer, which may be mediated through associations with specific signaling proteins in membranes [25]. Flaxseed oil FSO contains 50-60% omega-3 fatty acids in the form of ALA and this amount are roughly double that contained in FO. However, the results obtained in this study using FSO in the form of enema showed that it didn't provide any significant protective effect against AA-inducing colonic injury. This may be attributed to the fact that ALA present in FSO is a precursor and has to be converted in vivo into two major metabolites; the long chain omega-3 fatty acids, eicosapentaenoic acid EPA and docosahexaenoic acid DHA [18], such conversion require systemic and not local treatment. Therefore, further investigations are needed to explore the effect of changing the route of administration of FSO using oral feeding.

CONCLUSION:

The results presented in this article reflect that FO but not FSO is beneficial in the prevention of UC induced by AA when given in the form of enema. This data reinforce the previous reports on the beneficial effects of omega-3 rich compounds on IBD. However, we could not completely rule out any beneficial effects of FSO in UC until further investigations using the oral route of administration are completed.

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