The Dual Modulatory Effect of Folic Acid Supplementation on Indomethacin Induced Gastropathy in the Rat

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Abstract: Folic acid modulates several disorders in humans, including gastrointestinal inflammation at the basal requirement supplemental dose. In the present study we investigated upon the effects of folic acid supplementation at varying doses on indomethacin induced gastric ulceration in the rat. Prior to ulcer induction by indomethacin, animals in the experimental groups were treated with 1 mg/kg, 2 mg/kg and 3 mg/kg diet of folic acid for twenty-one (21) days. Following sacrifice, four hours after the formation of ulcers, severity of gastric lesions was assessed and colorimetric assays were then applied to determine the concentration of mucus, malondialdehyde (MDA), activities of catalase (CAT) and superoxide dismutase (SOD) in homogenized gastric mucosal samples. Indomethacin caused severe damage to the glandular portion of rats’ stomach with increase in MDA concentration, reductions in mucus, CAT and SOD concentration (p<0.001). Folic acid supplementation at 2 mg/kg diet reduced significantly the formation of gastric lesions by indomethacin, while at 3 mg/kg potentiation of the lesions was observed (p<0.05). On the other hand, MDA concentration is significantly decreased and SOD activity increased in the 2 mg/kg folic acid pre-treated group (p<0.05) while in the 3 mg/kg folic acid pre-treated significant increase in the MDA concentration and decrease in activities of both CAT and SOD were observed. There was a marked increase in the mucus concentration in 2 mg/kg folic acid pretreated, but decrease in 3 mg/kg diet of folic acid when compared with control. Pre-treatment with 1 mg/kg diet of folic acid revealed no significant changes. Histopathological studies underlined differences on the indomethacin-induced alterations in gastric mucosal structure on pre-treatment with 2 mg/kg and 3 mg/kg folic acid. This study revealed that folic acid is gastroprotective at the basal requirement supplemental dose; high folic acid supplementation may be dangerous to the integrity of the stomach.

Key words: Folic acid · Indomethacin · Ulcer · Malondialdehyde · Superoxide dismutase · Catalase

INTRODUCTION

Folate is a water-soluble vitamin essential for cell replication and DNA synthesis, repair and methylation [1]. Folic acid plays an important role in the pathophysiology of several disorders in humans including macrocytic anaemia, cardiovascular diseases [2], thromboembolic processes [3], neural tube and congenital defects [4], adverse pregnancy outcomes [5] and neuropsychiatric disorders [6]. Supplementation with folic acid reduces the risk of congenital heart defects, cleft palate, limb defects, urinary tract anomalies, neural tube defects and chronic liver diseases [7-10]. It is, also, a safe and effective supplement that may prevent isolated systolic hypertension [11] and several malignancies including cancer of the colorectum, lungs, pancreas, oesophagus, stomach, cervix, breast [12, 13] and neuroblastoma and leukaemia [14, 15]. Some data have equally indicated that folic acid may play an important role in the chemoprevention of gastric carcinogenesis by enhancing gastric epithelial apoptosis in the patients with premalignant lesions [16].

The gastroprotective activity of folic acid supplementation at the basal requirement supplemental dose of 2 mg/kg diet against the lipid peroxidative activity of indomethacin was mentioned [17] and the most commonly used model for experimental ulceration is oral administration of indomethacin, a non-steroidal anti-inflammatory drug.

Indomethacin induces an injury to gastrointestinal mucosa in experimental animals and humans and their use is associated with a significant risk of hemorrhage, erosions and perforation of both gastric and intestinal ulcers [18].
Folic acid supplementation is believed to be safe and free of toxicity [19], it may demonstrate some undesirable effects especially in some population not targeted for the dietary fortification [15]. For instance, clinical and experimental studies suggest that folate possesses dual modulatory effects on carcinogenesis, depending on the timing and intervention dose [20]. Therefore, the purpose of this study was to examine whether the anti-oxidative property of folic acid supplementation on gastric mucosa earlier reported will be dose dependent or not. This will further descriptively and/or pharmacologically evaluate the effects of folic acid on indomethacin induced gastropathy.

**MATERIALS AND METHODS**

**Animals:** Forty-two male albino rats: Wister strain weighing between 180-250 g were used for the present study. The animals were obtained from the Animal House of Igbinedion University, Okada and then, separated randomly into six wire meshed cages with seven rats each and kept for four weeks before the commencement of the experiment. The animals were housed under standard conditions of temperature (23±2°C), humidity (55±15%) and 12 hour light (7.00am- 7.00pm). The cages were constantly kept hygienically clean in order to prevent the animals from disease. They were fed with standard commercial rat pellets (Ladokun Feeds Limited, Nigeria) and allowed water *ad libitum*.

**Drugs:** Folic acid tablets and indomethacin were obtained from a local pharmacy duly registered by the Pharmacists’ Council of Nigeria (PCN). All other reagents were of analytical grade and obtained from British Drug Houses, Poole, UK.

**Experimental Design:**

**Grouping.**
The Animals Were Divided into Six Groups of Seven Rats Each.

**Group One:** Animals were kept as non-treated control group.

**Group Two:** Animals were treated with indomethacin (25 mg/kg b.w) after 24 hour fasting; treated control group.

**Group Three:** Animals were treated with 1 mg/kg diet of folic acid for three weeks before indomethacin administration.

**Group Four:** Animals were treated with 2 mg/kg diet of folic acid for three weeks before indomethacin administration.

**Group Five:** Animals were treated with 3 mg/kg diet of folic acid for three weeks before indomethacin administration.

**Group Six:** Animals received Ranitidine (4 mg/kg b.w) prior to indomethacin administration. This group serves as the positive control group.

Folic acid and indomethacin were administered orally and the animals were sacrificed under sodium pentobarbitone anaesthesia. The Central Animal Facility/Ethics Committee of Igbinedion University, Okada approved the experimental protocols.

**Ulcer Induction and Index Determination:** Four hours after the oral administration of indomethacin the stomachs were opened along the greater curvature, washed in normal saline to remove debris and pinned on a cork mat for ulcer scoring. This was done by locating the wounds in the glandular region under a simple microscope. The length (mm) of all the elongated black-red lines parallel to the long axis of the stomach in the mucosa was measured. Index of ulceration was calculated as the total lesion lengths divided by the number in each group [21].

**Biochemical Analysis:**

**Determination of Malondialdehyde (MDA):** The assay method of Hunter et al [22], modified by Gutteridge and Wilkins [23] was adopted. Malondialdehyde (MDA), a product of lipid peroxidation, when heated with 2-thiobarbituric acid (TBA) under acid conditions forms a pink colored product which has a maximum absorbance of 532nm. The stomach homogenate was supplemented with 1 g of TBA in 100 ml of 0.2% NaOH and 3 ml of glacial acetic acid, thoroughly mixed incubated in boiling water bath for 15 minutes, then allowed to cool after which they were centrifuged. Absorbance was read at 532nm and the results expressed as nanomoles MDA/mg wet tissue.

Determination of Catalase activity: Activity of catalase in gastric mucosa was determined according to the procedure of Sinha [24]. This method is based on the reduction of dichromate in acetic acid to chromic acetate when heated in the presence of H$_2$O$_2$, with the formation of perchromic acid as an unstable intermediate. The chromic acetate so produced is measured. Absorbance was read at 480nm within 30-60 seconds against distilled water.
**RESULTS**

**Development of Gastric Lesions:** Indomethacin caused severe damage to the stomachs of the rats (p<0.05) (Table 1).

Pre-treatment of rats with 1 mg/kg diet of folic acid produced no significant change in the formation of ulcers by indomethacin. For animals treated with 2 mg/kg diet of folic acid before ulcer induction by indomethacin, there was a reduction in the status of ulceration when compared with the ulcer control group (p<0.05). However, there was an increase in the status of the ulcer formed in the animals pre-treated with 3 mg/kg diet of folic acid when compared with control group (p<0.05) (Table 1).

**Gastric Mucus Concentration:** Gastric mucus was depleted significantly in the indomethacin treated group when compared with the normal saline group. Pre-treatment with 1 mg/kg and 3 mg/kg folic acid led to further decrease in the mucus concentration while 2 mg/kg caused a marked increase (p<0.05) (Table 1).

**Lipid Peroxidation and Anti-Oxidative Enzymes:** Fig. 2 [a, b and c] showed the lipid peroxidation (MDA), catalase and superoxide dismutase activities in both the normal and ulcerated gastric mucosa. Lipid peroxidation is measured as the amount of TBARs in the gastric mucosa, the results were expressed as MDA formed using an extinction coefficient of 1.56 X 10^5 Mcm. Indomethacin produced lipid peroxidation in the normal mucosa (p<0.05). In the same vein, the superoxide dismutase activities reduced significantly (p<0.05) while the catalase activity remained unchanged (p>0.05). Pre-treatment with 1 mg/kg diet of folic acid caused no significant change in MDA concentration, CAT and SOD activities when compared with the treated control. Pre-treatment with 2 mg/kg diet of folic acid reduced the MDA concentration and increased the activity of superoxide dismutase.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Gastric Mucus (mg/g) (Mean ± SEM)</th>
<th>Ulcer score (mm) (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Distilled water</td>
<td>35.50±0.90</td>
<td>0.00</td>
</tr>
<tr>
<td>II</td>
<td>Indo only (25 mg/kg)</td>
<td>30.25±0.25⁺</td>
<td>26.80±4.59⁺</td>
</tr>
<tr>
<td>III</td>
<td>1 mg/kg Folic Acid + Indo (25 mg/kg)</td>
<td>26.10±0.20⁺⁺</td>
<td>27.40±5.36⁺</td>
</tr>
<tr>
<td>IV</td>
<td>2 mg/kg Folic Acid + Indo (25 mg/kg)</td>
<td>34.50±0.30⁺⁺</td>
<td>20.60±4.06⁺</td>
</tr>
<tr>
<td>V</td>
<td>3 mg/kg Folic Acid + Indo (25 mg/kg)</td>
<td>24.50±0.60⁺⁺</td>
<td>35.20±4.14⁺⁺</td>
</tr>
<tr>
<td>VI</td>
<td>Ranitidine (4mg/kg) + Indo (25 mg/kg).</td>
<td>33.25±0.10⁺⁺</td>
<td>13.55±2.10⁺⁺</td>
</tr>
</tbody>
</table>

Indomethacin: Highly significant from distilled water, ⁺p<0.001, significant p<0.05. Folic acid and Ranitidine: Significant from indomethacin treated, ⁺⁷⁺⁺p<0.05
Fig. 2 a, b and c: Effect of folic acid on MDA concentration, CAT and SOD activities in gastric mucosa. Each vertical represents mean ± SEM of seven rats each. **P<0.001 (c.f Distilled water), a,b,c p<0.05 (c.f Indo only)
Photomicrographs of gastric mucosa sections from NS (normal stomach) and INDO (ulcerated stomach)
NS: Cells are seen in normal position. INDO: Disrupted mucosa and degenerated cells observed, degenerating layer of the muscularis mucosae. Stain; Haematoxylin-eosin. Magnification; X40

Photomicrographs of gastric mucosa sections from INDO, 1 FA (1 mg/kg folic acid), 2 FA (2 mg/kg) and 3 FA (3 mg/kg) groups.
INDO: Disrupted mucosa and degenerated cells observed, degenerating layer of the muscularis mucosae. 1 FA: Disoriented cells, necrosis seen, gastric glands appear inflamed. 2 FA: gastric gland, pits seen, exfoliated cells, layer of the muscularis mucosae appear unaffected. 3 FA: Disrupted mucosa, degenerated/exfoliated cells, congested blood vessels apparent beneath the degenerated layer of muscularis mucosae. Stain; Haematoxylin-eosin. Magnification; X40
Pre-treatment with 3 mg/kg diet of folic acid increased MDA concentration, decreased CAT and SOD activities when compared with the treated control (p<0.05).

Although 2 mg/kg of folic acid inhibited the development of ulcer by 23.1% ranitidine afforded a 51.9% protection on the mucosa, with an increase the mucus production (p<0.05) (Table 1). In the same vein, it reduced MDA concentration and increased the activities of both catalase and superoxide dismutase (p<0.05).

**DISCUSSION**

The findings of the present study confirmed that folate is an important factor in the *de novo* synthesis of purines and thymidine, DNA stability and apoptosis [28] and delayed the development of gastric ulcer only at the basal requirement dose. The molecular basis for the gastrointestinal toxicity of NSAIDs is widely believed to their inhibitory activity against cyclooxygenase, which causes them to block the production of prostaglandins and their therapeutic actions. Suppression of prostaglandin synthesis is associated with reduction of gastric mucosal blood flow, disturbance of microcirculation, decrease in mucus secretion, lipid peroxidation and neutrophil activation, which are involved in the pathogenesis of gastrointestinal mucosal disorders [29, 30].

The effects of folic acid dietary manipulation have been extensively studied in experimental models of cancer and cardiovascular disease [15], but there is still a relative paucity of data regarding the effects of folic acid supplementation on gastrointestinal inflammatory disorders.

Gastrointestinal wall integrity is known to be controlled by two opposing forces: aggressive and the defensive [27]. The aggressive force encompasses the increase in acid output and subsequent lipid peroxidation, which is a result of the reaction between oxyradicals and the polyunsaturated fatty acids. The defensive are gastro protective and involve the anti-oxidative enzymes; superoxide dismutase (SOD) which catalyses the dismutation of superoxide radical anion (O$_2^-$) into less noxious hydrogen peroxide (H$_2$O$_2$) and catalase (CAT) or glutathione peroxidase that inactivate H$_2$O$_2$ to water [31]. Indomethacin has been known to cause lipid peroxidation [32, 33] with depletion of endogenous antioxidant. In the present study, this is confirmed by the decrease in the activities of both catalase (CAT) and superoxide dismutase (SOD) with the concomitant increase in malondialdehyde (MDA) concentration in the homogenized gastric mucosal samples after indomethacin administration. Depletion of the antioxidant reserve and mucus in the gastric mucosa is an important factor in the pathogenesis of peptic ulceration. Hence, increase in the superoxide dismutase activity and mucus concentration observed in the group of ulcerated animals pre-treated with 2 mg/kg folic acid portend somewhat gastro protective tendencies of folate (at this dose), because they are scavengers which mop up and resist free radicals predisposing the stomach to inflammation. Moreover, this is underscored by the decrease in the MDA concentration observed in this group of animals which agrees with the mild severity of the wound in the glandular portion of the stomach when viewed macromorphologically and assessed histopathologically. The implication of this could mean that folate inhibits the lipid peroxidation activity of indomethacin. These may not be in dissension with the earlier reports of Cao et al. [16] which demonstrated that both the epithelial apoptosis rate and the tumor suppressor p53 expression in gastric mucosa were significantly increased, while the expression of Bcl-2 oncogene protein decreased after folic acid treatment of patients with premalignant gastric lesions.

However, the pre-treated ulcerated animals with 1 mg/kg diet of folic acid showed no remarkable difference either macromorphologically or biochemically. It may, therefore, be suggested that antioxidant or gastro protective tendencies of folate may be found wanting when administered at subnormal dose.

Animals pre-treated with 3 mg/kg diet of folic acid showed an increase in the MDA concentration with reduced activities of both catalase (CAT) and superoxide dismutase (SOD), supported by more severe wounds in the glandular portion of the stomach.

Folic acid has been shown to possess dual modulatory role, depending on the dose and timing of intervention in disease states [13], which is critical in providing safe and effective chemoprevention. Also, Marsillach et al. [34] demonstrated that moderately high folic acid supplementation exacerbates experimental induced liver fibrosis in rats. This could interpret to the fact that under certain clinical conditions, (moderately) high folic acid supplementation can have undesirable effects.

Conclusively, folic acid supplementation is ameliorative on indomethacin-induced gastric ulceration at the basal requirement supplemental level. While low folic acid may have no effect, high folic acid potentiates gastric ulceration.
REFERENCES


