Do Desipramine [10,11-Dihydro-5-[3-(methylamino) propyl]-5H-dibenz[b,f]azepine monohydrochloride] and Fluoxetine [N-Methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]-propan-1-amine] Ameliorate the Extent of Colonic Damage Induced by Acetic Acid in Rats?

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ABSTRACT

The present study was designed to compare the anti-inflammatory and antioxidant effects of two antidepressant drugs, desipramine [10,11-dihydro-5-[3-(methylamino) propyl]-5H-dibenz[b,f]azepine monohydrochloride] and fluoxetine [N-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]-propan-1-amine], administered with variable doses, on experimentally induced colitis in rats. Two doses for each drug (10 and 20 mg/kg/day i.p.) were injected in 48 adult male albino rats for 2 weeks after induction of colitis by intracolonic administration of 2 ml of 3% acetic acid. Several parameters, including macroscopic (ulcer score index) and biochemical such as myeloperoxidase (MPO), reduced glutathione (GSH), tumor necrosis factor (TNF)-α, and interleukin (IL)-1β, were measured using standard assay procedures. The study demonstrates that both desipramine and fluoxetine significantly attenuated the extent and the severity of the macroscopic signs of cell damage. Both drugs significantly reduced tissue MPO activity in a dose-dependent manner. Both desipramine and fluoxetine, at either dose, significantly increased GSH in colonic tissue. Desipramine and fluoxetine, at either dose, significantly reduced TNF-α and IL-1β. Desipramine at the dose of 20 mg/kg produced more decrease in the level of TNF-α compared with the effect of the smaller dose, but fluoxetine at 10 mg/kg diminished more in the level of IL-1β compared with the effect of the larger dose. The present data indicate that both desipramine and fluoxetine have anti-inflammatory and antioxidants effects in experimentally induced colitis in rats, opening the avenue to their possible protective role in patients with inflammatory bowel disease.

Ulcerative colitis (UC) is a chronic inflammatory disorder of the large bowel; the disease typically starts in the rectum but often extends to involve length of colon (Podolsky, 2002). It is an ulceroinflammatory disease; the ulcer is limited to the mucosa and submucosa, except in severe cases (Gionchetti et al., 2005). In some patients, it is associated with extraintestinal manifestations involving the liver, skin, eyes, and/or joints (Jakobovits and Travis, 2006). It is a worldwide disorder with significant geographical heterogeneity (Sood et al., 2003). It is common in the United States and western countries, with an incidence of seven per 100 in population, with a peak incidence between ages 20 and 25 years. There is limited information about the inflammatory bowel disease (IBD) in the Arab community (Yang et al., 2001). UC is still of unknown etiology. However, the interaction of environmental factors with genetic susceptibility and immune-mediated phenomena may play important roles (Olden, 2002). Treating UC using limiting drug-induced toxicity is a continuous challenge. The 5-aminosalicylic acid, corticosteroids, azathioprine, 6-mercaptopurine, methotrexate, cyclosporine, antibiotics (e.g., metronidazole, ciprofloxacin, and vancomycin), and the TNF antagonist, infliximab, are the main current medical treatments (Chang and Cohen, 2004). Because of the lack of specific, curative treatment with limited toxicity, there is a pressing need for the developing of effective therapeutic approaches. Until globally effective
treatment is found, there is a need to decrease the number of relapses of the disease, lengthen remission, and improve quality of life and psychosocial functioning of patients.

Recently, there is a great suggestion that psychiatric disorders could be one of the etiological factors in some patients with UC (Kurina et al., 2001). There are several possible explanations for the co-occurrence of depression or anxiety and IBD (Addolorato et al., 1997). There is some evidence that major depression in particular is accompanied by activation of the inflammatory system and that proinflammatory cytokines may play a role in the etiology of depression (Papadakis and Targan, 2000). Kubera et al. (2001a) reported that systemic administration of proinflammatory cytokines such as IL-1-induced changes in the endocrine and central monoamine systems that are reminiscent of those observed in depression; thus, these proinflammatory cytokines may conceivably contribute to the etiology of depression (Kubera et al., 2001b). Indeed, some depressed patients exhibit increased TNF-α levels, which normalize upon treatment with antidepressants. It remains unclear whether this relationship reflects a causal role for depression in susceptibility to inflammatory stimuli or a common inflammation-based etiopathology (Anisman and Merali, 2003).

The exact mechanisms by which antidepressant drugs affect cytokine production still remain to be elucidated. Among antidepressant drugs is desipramine, which is the metabolite of imipramine; both of them are widely described tricyclic antidepressant drugs. It has been shown that imipramine increases the production of IL-6 (Kubera et al., 2004) and IL-10 (Kubera et al., 2001b). In addition to its effects on cytokines, it was found that after chronic administration, imipramine decreases the expression of corticoid-releasing hormone messenger RNA in the hypothalamus (Brady and Whitefield, 1991). Other antidepressant drugs are selective serotonin reuptake inhibitors (SSRIs) that have a well-recognized effect on depression and anxiety (Addolorato et al., 1997). Researchers have also proposed that SSRIs have an antidepressant effect (Abdel-Salam et al., 2004). Fluoxetine is an SSRI of proven efficacy in major depressive disorders. It significantly enhances the IL-10 production and significantly suppresses the interferon-γ/IL-10 ratio (Kubera et al., 2001a). It has been postulated that because cytokines play a role in the pathophysiology of depression, antidepressant treatment might be effective, in part, by interfering with cytokine production and activity. The main aim of the present study was to assess and compare the possible therapeutic effects of desipramine and fluoxetine on the extent and severity of colitis induced by acetic acid in rats and also to know the possible underlying mechanisms of action by which the antidepressant drugs emerged their effects.

Materials and Methods

Forty-eight adult male albino rats (150–200 g) were used throughout this work. The animals were maintained under standard condition of light, feeding, and temperature at the animal house of the Pharmacology Department, Alexandria Faculty of Medicine. All animal experiments were conducted after the consent of the Faculty Ethical Committee.

The rats were divided randomly into six groups (n = 8) as follows: group 1, normal control group that was sham-operated and received saline in a dose of 0.5 ml/kg/day i.p. for 2 weeks after sham operation; group 2, acetic acid-induced colitis (AAIC) (done as described below) untreated control group that received saline in a dose of 0.5 ml/kg/day i.p. for 2 weeks after AAIC; group 3, AAIC desipramine-treated group that received desipramine in a dose of 10 mg/kg i.p. for 2 weeks after AAIC (Abdel-Salam et al., 2004); group 4, AAIC desipramine-treated group that received desipramine in a dose of 20 mg/kg i.p. for 2 weeks after AAIC (Abdel-Salam et al., 2004); group 5, AAIC fluoxetine-treated group that received fluoxetine in a dose of 10 mg/kg i.p. for 2 weeks after AAIC (Abdel-Salam et al., 2003); and group 6, AAIC fluoxetine-treated group that received fluoxetine in a dose of 20 mg/kg i.p. for 2 weeks after AAIC (Abdel-Salam et al., 2003).

Doses of antidepressant drugs used in the present study were chosen based on stated references reporting documented anti-inflammatory effect of either drug. Desipramine HCl was purchased from Sigma-Aldrich (St. Louis, MO). Fluoxetine HCl was obtained from Eli Lilly & Co. (Indianapolis, IN).

Induction of Experimental Colitis in Rats (Millar et al., 1996)

The animals were starved for 24 h with access to water ad libitum before induction of colitis. Each rat was sedated by injection of phenobarbitone (35 mg/kg i.p.). An infant feeding tube (2-mm o.d.; Pennine Healthcare, Derbyshire, UK) was inserted into the colon to 8 cm distal to the anus and 2 ml of acetic acid [3% (v/v) in 0.9% saline] was infused into the colon. The acetic acid was retained in the colon for 30 s, after which fluid was withdrawn. At the end of the experimental period, blood samples were collected, and rats were decapitated. A segment of colon 10 cm in length, extending proximally 2 cm above the anal margin, was removed and processed to measure the studied parameters.

Assessment of Colitis

Macroscopic Scoring. Colonic mucosal lesions were scored on the scale (ranging from 0–5) adapted from Morris et al. (1989).

Biochemical Study. Colonic samples were stored immediately at −20°C until analysis. Tissue samples were homogenized in 1 ml of 10 mM Tris-HCl buffer, pH 7.1, and homogenates were used for measurement of myeloperoxidase activity (Bradley et al., 1982) and reduced GSH concentration (Owens and Bechsler, 1965).

From the blood samples, sera were separated and used for measurement of concentration of TNF-α (Beuler, 1995) and IL-1β (Brissow et al., 1991). Circulating cytokine levels were measured 2 weeks after onset injury because this was meant to be a chronic study. TNF-α was measured using commercially available rat enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN) and absorbance was read at 450 nm. Concentration of IL-1β was measured using commercially available rat enzyme-linked immunosorbent assay kits (R&D Systems), and absorbance was read at 540 nm.

Statistical Analysis

All data were expressed as mean ± S.E.M. for eight rats per experimental group. Statistical group analysis was performed with SPSS version 10.0 statistical software (SPSS Inc., Chicago, IL). One-way analysis of variance was used to compare the mean values of quantitative variable among the groups. Bonferroni’s test was used to identify the significance of pairwise comparison of mean values among the groups. Statistically significant differences were accepted at P < 0.05.

Results

Macroscopic Scoring. The acetic acid treatment induced severe macroscopic inflammation in the colon as assessed from the colonic damage score. Treatment with desipramine or fluoxetine significantly reduced the severity of gross lesion score in a dose-dependent manner (Table 1).
Myeloperoxidase Activity. Treatment with different doses of desipramine (10 or 20 mg/kg) or fluoxetine (10 or 20 mg/kg) after induction of colitis significantly decreased colonic myeloperoxidase activity as compared with the AAIC control group (Table 2).

Reduced GSH Concentration. Colonic GSH concentration was significantly decreased after induction of colitis as compared with the normal control group \((P < 0.001)\). After treatment with the studied drugs for 2 weeks, there was a significant increase of colonic GSH concentration as compared with AAIC control (Table 3).

Tumor Necrosis Factor-\(\alpha\) Concentrations. TNF-\(\alpha\) concentration in serum of rats was increased significantly in the inflamed colon 2 weeks after acetic acid administration in comparison with the normal control \((P < 0.001)\). Administration of either desipramine (10 or 20 mg/kg) or fluoxetine (10 or 20 mg/kg) resulted in a significant reduction in serum TNF-\(\alpha\) concentration in comparison with the AAIC control \((P < 0.001)\). Desipramine in a dose of 20 mg/kg significantly decreased the level of serum TNF-\(\alpha\) concentration in comparison with desipramine in a dose of 10 mg/kg or fluoxetine either in doses of 10 or 20 mg/kg (Table 4).

Interleukin-1\(\beta\) Concentrations. IL-1\(\beta\) level was increased significantly in all groups after induction of ulcerative colitis in comparison with the normal control \((P < 0.001)\). Administration of variable doses of either desipramine (10 or 20 mg/kg) or fluoxetine (10 or 20 mg/kg) led to a significant decrease of serum IL-1\(\beta\) concentration in comparison with the AAIC control \((P < 0.001)\). Administration of 10 mg/kg fluoxetine decreased significantly the level of serum IL-1\(\beta\) concentration, in comparison with other groups tested with 10 or 20 mg desipramine and 20 mg/kg fluoxetine \((P < 0.001)\) (Table 5).

### Table 2

<table>
<thead>
<tr>
<th>No.</th>
<th>Group</th>
<th>MPO Units/g Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>0.41 ± 0.027</td>
</tr>
<tr>
<td>2</td>
<td>Acetic acid control</td>
<td>1.01 ± 0.025*</td>
</tr>
<tr>
<td>3</td>
<td>10 mg/kg Desipramine</td>
<td>0.59 ± 0.024**</td>
</tr>
<tr>
<td>4</td>
<td>20 mg/kg Desipramine</td>
<td>0.46 ± 0.023**</td>
</tr>
<tr>
<td>5</td>
<td>10 mg/kg Fluoxetine</td>
<td>0.70 ± 0.026**</td>
</tr>
<tr>
<td>6</td>
<td>20 mg/kg Fluoxetine</td>
<td>0.52 ± 0.021**</td>
</tr>
</tbody>
</table>

\* \(P < 0.001\) in comparison with group 1 normal control.
\* \(P < 0.001\) in comparison with group AAIC control group.

### Table 3

<table>
<thead>
<tr>
<th>No.</th>
<th>Group</th>
<th>GSH mg/g Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>1436 ± 57</td>
</tr>
<tr>
<td>2</td>
<td>Acetic acid control</td>
<td>639 ± 46*</td>
</tr>
<tr>
<td>3</td>
<td>10 mg/kg Desipramine</td>
<td>805 ± 74*</td>
</tr>
<tr>
<td>4</td>
<td>20 mg/kg Desipramine</td>
<td>813 ± 74*</td>
</tr>
<tr>
<td>5</td>
<td>10 mg/kg Fluoxetine</td>
<td>781 ± 19**</td>
</tr>
<tr>
<td>6</td>
<td>20 mg/kg Fluoxetine</td>
<td>750 ± 20**</td>
</tr>
</tbody>
</table>

\* \(P < 0.001\) in comparison with group 1 normal control.
\* \(P < 0.001\) in comparison with group AAIC control group.

### Table 4

<table>
<thead>
<tr>
<th>No.</th>
<th>Group</th>
<th>TNF-(\alpha) ng/ml Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>1.47 ± 0.021</td>
</tr>
<tr>
<td>2</td>
<td>Acetic acid control</td>
<td>13.92 ± 0.28*</td>
</tr>
<tr>
<td>3</td>
<td>10 mg/kg Desipramine</td>
<td>6.63 ± 0.16*</td>
</tr>
<tr>
<td>4</td>
<td>20 mg/kg Desipramine</td>
<td>3.73 ± 0.17*</td>
</tr>
<tr>
<td>5</td>
<td>10 mg/kg Fluoxetine</td>
<td>9.32 ± 0.14*</td>
</tr>
<tr>
<td>6</td>
<td>20 mg/kg Fluoxetine</td>
<td>7.71 ± 0.18*</td>
</tr>
</tbody>
</table>

\* \(P < 0.001\) in comparison with group 1 normal control.
\* \(P < 0.001\) in comparison with group AAIC control group.

### Table 5

<table>
<thead>
<tr>
<th>No.</th>
<th>Group</th>
<th>IL-1(\beta) pg/ml Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>50.54 ± 0.66</td>
</tr>
<tr>
<td>2</td>
<td>Acetic acid control</td>
<td>577.13 ± 5.85*</td>
</tr>
<tr>
<td>3</td>
<td>10 mg/kg Desipramine</td>
<td>493.23 ± 1.83*</td>
</tr>
<tr>
<td>4</td>
<td>20 mg/kg Desipramine</td>
<td>422.12 ± 5.17*</td>
</tr>
<tr>
<td>5</td>
<td>10 mg/kg Fluoxetine</td>
<td>275.03 ± 15.05*</td>
</tr>
<tr>
<td>6</td>
<td>20 mg/kg Fluoxetine</td>
<td>371.36 ± 7.44*</td>
</tr>
</tbody>
</table>

\* \(P < 0.001\) in comparison with group 1 normal control.
\* \(P < 0.001\) in comparison with group AAIC control group.

### Discussion

The causes of IBD are still unknown. For decades, some clinicians have held the view that IBD, particularly UC, may be psychosomatic or psychoneuroimmunological conditions (Kurina et al., 2001). There are several possible explanations for the co-occurrence of depression or anxiety and IBD. The two psychiatric disorders might predispose people to IBD or, conversely, IBD might predispose people to depression or anxiety. IBD and the psychiatric disorders might share a common environmental, behavioral, or genetic etiology (Addolorato et al., 1997). Recently, it was demonstrated that major depression is related to activation of the inflammatory response. The evidence includes, among other things, an enhanced production of proinflammatory cytokines such as IL-1\(\beta\) and IL-6 (Maes, 1999).

The present study demonstrated that treatment of rats with desipramine and fluoxetine at a dose of 10 or 20 mg/kg i.p. administered for 2 weeks after induction of colitis reduced the inflammation and the acute colonic damage induced by acetic acid as verified by macroscopic and biochem-
ical data. Our data showed that desipramine and fluoxetine have anti-inflammatory effect because both drugs, at different doses, significantly reduced the serum levels of TNF-α and IL-1β and colonic myeloperoxidase activity. Moreover, desipramine at the large dose, 20 mg/kg, produced more decrease in the level of TNF-α than the lower dose and the variable doses of fluoxetine. Paradoxically, fluoxetine at a low dose, 10 mg/kg, decreased the level of IL-1β more than the larger dose of fluoxetine and the other doses of desipramine. This paradoxical finding might be due to some irritant effect of large doses of fluoxetine on the gastrointestinal tract.

In previous experimental studies, TNF-α and IL-1β were reported to produce epithelial cell necrosis, edema, neutrophil infiltration, and global cell depletion (Stallmach et al., 1992). Blocking the action of endogenous TNF-α and IL-1β attenuates acute and chronic experimental colitis and its systemic complications (Eigler et al., 1997; Ludwiczek et al., 2004). IL-1β stimulates anion secretion by epithelial cells indirectly through the liberation of prostaglandins. Also, IL-1β augments hydrogen peroxide-, bradykinin-, and histamine-induced epithelial chloride secretion. Thus, IL-1β appears to be one of the primary stimulators of tissue damage in IBD (Kubera et al., 2005).

Our macroscopic and biochemical results showed a significant reduction of the level of these cytokines, which may be explained by inhibition of their synthesis, production, and release or inhibition of their biological activity. Other studies explained the anti-inflammatory effects of antidepressants. It was published that ex vivo studies show that antidepressants inhibit the secretion of IL-1β and TNF-α and that they inhibit the proliferative activity of T cells and the cytotoxic activity of natural killer cells (Stallmach et al., 1992). Indeed, prolonged treatment of depressed patients with antidepressants has been found to be accompanied by a normalization of the initially increased production of IL-6 and positive acute-phase proteins, suggesting that the antidepressants have in vivo anti-inflammatory effects (Shuzewska et al., 1995). The antiinflammatory effect of these drugs might be mediated by cyclic adenosine monophosphate (Diamond et al., 2006). Other researchers proved that acute administration of a single dose of imipramine impairs the ability of macrophages to produce TNF-α after an in vivo challenge with bacterial lipopolysaccharide in rats (Dredge et al., 1999). Abdel-Salam et al. (2004) suggested that fluoxetine alone or either with imipramine or melatonin would be of benefit in inflammatory or neuropathic painful conditions. Moreover, they proved that imipramine, amitriptyline, cloimipramine, trazodone, and fluoxetine have anti-inflammatory and antinociceptive effects on the carrageenan paw edema and tail-electric stimulation assays in rats (Abdel-Salam et al., 2003).

Regarding the antioxidant effect of the studied drugs, the present study demonstrated that either fluoxetine or desipramine in doses of 10 and 20 mg/kg increased significantly the reduced glutathione concentration in ulcerative colonic tissue compared with nontreated control rats. The antioxidant effect of fluoxetine might be related to the fact that this drug increases the level of serotonin for which an antioxidant effect has been reported (Huether and Schuff-Werner, 1996). Desipramine has been shown recently to reduce mitochondrial reactive oxygen species and cell death by inhibiting membrane sphingomyelinase and ceramide production (Sue-matsu et al., 2003). Ceramide is the second messenger in the sphingomyelin signaling pathway. It has been shown to play an important role in mediating the cell apoptosis produced by TNF-α and oxygen free radicals (Cui et al., 2004). Desipramine has also been shown to reduce ceramide increase, infarct size, and myocyte apoptosis during prolonged ischemia in both isolated perfused hearts (Argaud et al., 2004) and intact rabbits (Corda et al., 2001).

In this respect, there is some controversy. Some studies demonstrated that a high concentration of amitriptyline and imipramine decreased intracellular GSH, whereas fluoxetine caused marked elevation of GSH levels in the rat gliona cells, which may be a protective effect of fluoxetine particularly (Slamon and Pentreath, 2000). In another study, it was found that desipramine significantly reverses the reduction in the activity of glutathione peroxidase in olfactory-bulbectomized rats (Song et al., 1994). Kolja et al. (2005) found that pretreatment with either amitriptyline or fluoxetine is associated with increased superoxide dismutase in cultured rat pheochromocytoma cells. Despite all the studies, the antioxidant effect of antidepressant drugs still remains for further investigation.

In addition, other mechanisms could account for the protective effect of antidepressants observed in the current study. Indeed, a recent study reported that experimental conditions that induced depressive-like behaviors in mice increased susceptibility to intestinal inflammation by interfering with the tonic vagal inhibition of proinflammatory macrophages and that tricyclic antidepressants restored vagal function and reduced intestinal inflammation (Ghia et al., 2008).

The results of the present study provided evidence of anti-inflammatory effects of antidepressant drugs, desipramine and fluoxetine, in induced ulcerative colitis in rats, which may shed new light on the underlying molecular mechanisms and pathophysiology of inflammatory bowel diseases with or without psychiatric disorders.

References


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