Effects of fluoxetine on mast cell morphology and protease-1 expression in gastric antrum in a rat model of depression

Zhen-Hua Chen, Ling Xiao, Ji-Hong Chen, He-Shen Luo, Gao-Hua Wang, Yong-Lan Huang, Xiao-Ping Wang

The average level of rMCP-1mRNA of gastric antrum significantly increased in depressed model group (0.759 ± 0.357 vs 0.476 ± 0.029, \( P < 0.01 \)) or saline + depressed model group (0.781 ± 0.451 vs 0.476 ± 0.029, \( P < 0.01 \)), while no significant difference was found between fluoxetine + normal control group (0.460 ± 0.027) or fluoxetine + depressed model group (0.488 ± 0.030) and normal control group. Fluoxetine showed partial inhibitive effects on mast cell ultrastructural alterations and de-regulated rMCP-1 expression in gastric antrum of the depressed rat model.

CONCLUSION: Chronic stress can induce mast cell proliferation, activation, and granule hyperplasia in gastric antrum. Fluoxetine counteracts such changes in the depressed rat model.

© 2008 The WJG Press. All rights reserved.

Key words: Depression model; Gastric antrum; Mast cell protease-1; Mast cells; Morphology; Fluoxetine hydrochloride

INTRODUCTION

Mast cells are now recognized as “granular cells of the connective tissue”, whose activation exacerbates allergic immune responses and as key players in the establishment of innate immunity as well as modulators of adaptive immune responses[1]. The role of mast cells in the gastrointestinal mucosa is not only to react to antigens, but also to actively regulate the barrier and transport properties of the intestinal epithelium. Mucosal mast cells respond to both IgE/antigen-dependent and non-IgE-dependent stimulation, releasing bioactive mediators into adjacent tissues where they induce physiological responses. Studies in models of hypersensitivity and stress
have provided evidence that changes in mucosal function are due to either direct action of mast cell mediators on epithelial receptors and/or indirect action via nerves/neurotransmitters[3]. Intestinal anaphylaxis is associated with disturbances in gut function that are antigen-specific and dependent on mast cell degranulation. During mucosal immunoglobulin E-mediated reactions, rat mast cell protease II (rMCP-II) is released and is associated with ultrastructural changes in the intestinal mucosa. The systemic appearance of this specific protease provides a serum marker of intestinal anaphylaxis. Psychological stress may trigger this sensitive alarm system via the brain-gut axis[8]. In clinical studies, it has become clear that psychological factors, especially anxiety and depression, play an important role in gastrointestinal diseases by precipitating exacerbation of symptoms[9,10]. Several studies have shown that the prevalence of chronic stress disorders in patients with gastrointestinal symptoms is 60%-85%[11,12]. Stress often worsens the symptoms of gastrointestinal diseases, which might be explained by altered neuroendocrine and visceral sensory responses to stress[9].

Fluoxetine hydrochloride (fluoxetine) is a kind of selective serotonin reuptake inhibitors (SSRIs), which belong to a class of antidepressants used in the treatment of depression and anxiety disorders. SSRIs increase the extracellular expression of the neurotransmitter serotonin by inhibiting its reuptake into the presynaptic cells. Studies have suggested that SSRIs may promote the growth of new neural pathways or neurogenesis[9]. SSRIs may also protect against neurotoxicity caused by other compounds as well as from depression itself. Recent studies showed that pro-inflammatory cytokine processes took place during depression in addition to somatic diseases and it was possible that symptoms manifested in these psychiatric illnesses were being attenuated by the pharmacological effects of antidepressants on the immune system[9]. SSRIs have been found to be immunomodulatory and anti-inflammatory against pro-inflammatory cytokine processes[10,11].

The aim of this study is to investigate the effects of fluoxetine hydrochloride (fluoxetine) on mast cell morphology and rMCP-1 expression in gastric antrum in a rat model of depression.

MATERIALS AND METHODS

Animals

Fifty healthy male Sprague-Dawley rats, weighing 250 ± 300 g, from the Animal Center, Hubei Academy of Preventive Medical Sciences, were used in the present study. The animals were fed standard rat chow, allowed access to tap water and acclimatized to the surroundings for 1 wk prior to the experiments.

Reagents

Cy3-conjugated goat anti-rabbit IgG, rMCP-1 rabbit anti-mouse antibody were purchased from Sigma Co., USA. Fluoxetine hydrochloride capsule was purchased from Lilly Co. Ltd. Other reagents used in the study were all of analytical grade.

Experimental protocols

All procedures were approved by the Animal Care Committee at the Medical Department of Wuhan University. A rat model of chronic stress-induced depression was established[13,14]. The rats received a variety of stressors for 21 d, including tail nip for 1 min, cold water swimming at 4°C for 5 min, heat stress at 45°C for 5 min, water deprivation for 24 h, food deprivation for 24 h, 12-h inverted light/dark cycle (7:00 a.m. lights off and 7:00 p.m. lights on), paw electric shock (electric current 1.0 mA10 s, every 1 min, lasting 10 s, 30 times). The animals were randomly divided into five groups (10 rats per group): normal control, fluoxetine + normal control, depressed model control, saline + depressed model, and fluoxetine + depressed model. The depressed animals were treated with saline and fluoxetine (10 mg/kg), respectively. A normal control group of rats without receiving any stress was included and housed in a separate room; food and water were freely available in their home cage.

Immunofluorescence histochemistry

The rats were anesthetized with urethane (5 mg/kg ip.) and rapidly killed by decapitation. The gastric antrum samples (1 cm × 1 cm) were perfused with 4% paraformaldehyde for immunofluorescence histology from each group. Each sample was cut into 30 sections and each section was cut 50-μm thick using a vibratome. Serial sections were placed on slides, three to a slide. The sections were numbered from 1 to 30. Ten sections were incubated. The staining procedure was as follows: (1) the sections were washed in phosphate-buffered saline (PBS), then pretreated with 0.25% Triton X-100 for 30 min at 37°C and rinsed in PBS; (2) incubation for 12 h at 4°C in a 1:100 dilution of the primary antibody of rMCP-1 in PBS; and (3) incubation with 1:200 diluted secondary antibody (Cy3-conjugated goat anti-rabbit IgG) in PBS for 1 h at 37°C. The sections were washed three times for 10 min after incubation steps 1 to 3, respectively, and were finally mounted in 50 g/L glycerin.

Detection was carried out according to the kit instructions (Leica SP2 TCS AOB5 Made in Germany). The specimens were excited with a laser beam at a wavelength of 492 nm (Cy3). The sections were observed under a laser scanning confocal microscope (LSCM) and analyzed with a Leica Q500IW image analysis system in terms of Cy3 fluorescent intensity.

Electron microscopic analysis

For electron microscopic analysis, gastric antrum tissue sections were fixed in modified Karnovsky’s medium containing 2% paraformaldehyde, 3% glutaraldehyde and 0.1% tannic acid in 0.1 mmol/L phosphate buffer (pH 7.4) and processed as before[15]. Each electron microscopic sample was divided into 5 blocks. Each block was cut into 10 sections (200 μm thick). Five sections selected
from 10 sections were observed. Ultrathin sections were placed onto copper grid, stained with uranyl acetate and lead citrate, and observed under a transmission electron microscope (Hitachi H-600, Japan). Mast cells were evaluated according to Letourneau[16]. Mast cells containing many intact electron-dense granules or containing empty granules were categorized as inactive and active cells, respectively. All mast cells were counted at magnification ×4000 in 30 visual fields.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)
To quantify the expression of rMCP-1 RNA, we performed a RT-PCR assay as described previously[17]. Total mRNA was isolated with TRIZOL (Invitrogen) according to the instructions of manufacturer. The primers for RT-PCR were as follows: rMCP-1 (237 bp), forward primer 5′-GCCTGTAAAAACTATTTT-3′; reverse primer 5′-CAGGCTGGTCAGATCCTGC-3′. GAPDH (217bp), forward primer 5′-GAAACCTGCATATGATGATG-3′; reverse primer 5′-ACCAGGAAATGAGCCTTGAGA-3′. The reaction mixture was added to the RNA solution and incubated at 42℃ for 1 h, heated at 94℃ for 5 min, and chilled at 4℃. For PCR, the cDNA reaction mixture was diluted with 40 ml of PCR buffer and mixed with 50 pmol of the primers. The reaction was carried out in a DNA thermal cycler under the following conditions: 94℃ for 30 s, 58℃ for 30 s and 72℃ for 45 s. Following the reaction, the amplified products were analyzed by 1.5% agarose gel electrophoresis and visualized using ultraviolet fluorescence after staining with ethidium bromide. The relative content of rMCP-1 mRNA was calculated densitometrically based on the densitometric ratio between rMCP-1 and GAPDH.

Statistical analysis
Data were expressed as mean ± SE. Statistical analysis was performed using one-way ANOVA and the non-parametric Mann-Whitney U test between groups. P values less than 0.05 were considered statistically significant.

RESULTS
Immunofluorescence histochemical assay
LSCM was used to prepare immunofluorescence picture, and LSCM imaging system was used to analyze the rMCP-1 immunofluorescence intensity among groups. Compared with the normal control group, the average immunofluorescence intensity of gastric antrum rMCP-1 significantly increased in depressed model group or saline + depressed model group (Figure 1, Table 1, \( P < 0.01 \)), while there was no significant difference between fluoxetine + normal control group or fluoxetine + depressed model group and normal control group. Compared with depressed model group, the average immunofluorescence intensity of gastric antrum rMCP-1 significantly decreased in fluoxetine + depressed model group (Figure 1, Table 1, \( P < 0.01 \)), while there was no significant difference between saline + depressed model group and normal control group. This confirmed that chronic stress induced mast cells to secrete rMCP-1 and fluoxetine inhibited this effect.

Ultrastructural morphology analysis
Compared with the normal control rats, the total number of mast cells/30 visual fields and the percentage of activated mast cells increased significantly, while the percentage of normal mast cells decreased significantly.
in chronic stress-induced depressed rats or saline + depressed model rats (Table 2, \( P < 0.01 \)). In depressed rats treated with fluoxetine, the changes in total number of mast cells/30 visual fields and the percentage of activated or normal mast cells were between normal control group and depressed model rats (Table 2, \( P < 0.05 \)).

In morphology, ultrastructural observations indicated that gastric antrum mast cells were rich and strictly in perivasculitis. In normal control rats or fluoxetine + normal control rats, the mast cells were spherical in shape with round nucleus, and contained electron-dense granules. Some secretary granules were intact with homogeneous electron dense content (Figure 2A and B). In chronic stress-induced depressed rats or saline + depressed rats, the mast cells were elongated with a fusiform nucleus, granules maldistributed and contained altered electron-dense content. The mast cells were proliferative, while the granules were also hyperplastic. Mast cell secretary granules exposed to the surface of the target and mast cells contained fibrillar material, empty granules and lipid bodies (Figure 2C and D). In depressed rats treated with fluoxetine, the morphological alterations were between normal control rats and depressed rats (Figure 2E).

**Effects of fluoxetine on gastric antrum rMCP-1mRNA by RT-PCR**

Compared with the normal control group, the average level of rMCP-1 mRNA of gastric antrum significantly increased in chronic stress-induced depressed model group or saline + depressed model group, while there was no significant difference between fluoxetine + normal control group or fluoxetine + depressed model group and normal control group. Compared with depressed model group, the average level of rMCP-1 mRNA of gastric antrum significantly decreased in fluoxetine + depressed model group, while there was no significant difference between saline + depressed model group and depressed model group (Figure 3, \( P < 0.01 \)).

**DISCUSSION**

Mast cells are immunocytes, which are widely distributed throughout the gastrointestinal tract. Several stimuli (e.g.
Mast cell activation has also been shown to occur in response to psychological stress. In addition, the effect of fluoxetine on mast cells: a possible link between psychological stress and mast cell activation. Mast cells are a type of immune cell that can be activated by stress, leading to release of inflammatory mediators such as histamine and serotonin. This activation can occur in response to acute or chronic stress.

Research frontiers
Some data strongly suggested that mast cells played an important role in pathophysiology of gastrointestinal diseases. Selective serotonin reuptake inhibitors (SSRIs) have been shown to be immunomodulatory and anti-inflammatory agents, which can ameliorate pathological changes in gastric antrum of depressed rats. These findings will conduce to understand that chronic stress may induce the immune responses in mast cells.

ACKNOWLEDGMENTS
We thank Dr. Shen-Lin Lei for his technical assistance.

REFERENCES
1. Stelekati E, Orinska Z, Bulfone-Paus S. Mast cells in allergy: innate instructors of adaptive responses. *Immunobiology* 2001; 212: 505-519
2. Yu LC, Perdue MH. Role of mast cells in intestinal mucosal function: studies in models of hypersensitivity and stress. *Immunol Res* 2001; 179: 61-73
3. Gui XY. Mast cells: a possible link between psychological stress and gastric mucosal integrity. *Immunol Rev* 2007; 212: 505-519

COMMENTS

**Background**
In clinical studies, it has become clear that depression plays an important role in gastrointestinal diseases by precipitating exacerbation of symptoms. The stress may induce mast cell activation and degranulation.

**Research frontiers**
Some data strongly suggested that mast cells played an important role in pathophysiology of gastrointestinal diseases.

**Innovations and breakthroughs**
The authors established a rat depression model, and observed the level of mast cell protease-1 (MCP-1) expression in the gastric antrum of depressed rats. Fluoxetine counteracted such stress effects on mast cell morphology and MCP-1 expression in the depressed rats.

**Applications**
These findings will conduce to understand that chronic stress may induce the immune responses in mast cells. Treatment with fluoxetine can ameliorate pathological changes in gastric antrum of depressed rats, suggesting that SSRIs are an effective therapeutic agent for some gastroduodenal diseases caused by psychological factors.

**Terminology**
Rat mast cell protease-1 (MCP-1) is released and is associated with mast cell activation and degranulation.

**Peer review**
The authors of the present study showed that depression led to mast cell activation and degranulation in the gastric antrum. Fluoxetine counteracted such changes in gastric antrum in the depressed rat model.

**REFERENCES**

1. Stelekati E, Orinska Z, Bulfone-Paus S. Mast cells in allergy: innate instructors of adaptive responses. *Immunobiology* 2001; 212: 505-519
2. Yu LC, Perdue MH. Role of mast cells in intestinal mucosal function: studies in models of hypersensitivity and stress. *Immunol Res* 2001; 179: 61-73
3. Gui XY. Mast cells: a possible link between psychological stress and gastric mucosal integrity. *Immunol Rev* 2001; 179: 61-73

**COMMENTS**

**Background**
In clinical studies, it has become clear that depression plays an important role in gastrointestinal diseases by precipitating exacerbation of symptoms. The stress may induce mast cell activation and degranulation.

**Research frontiers**
Some data strongly suggested that mast cells played an important role in pathophysiology of gastrointestinal diseases.

**Innovations and breakthroughs**
The authors established a rat depression model, and observed the level of mast cell protease-1 (MCP-1) expression in the gastric antrum of depressed rats. Fluoxetine counteracted such stress effects on mast cell morphology and MCP-1 expression in the depressed rats.

**Applications**
These findings will conduce to understand that chronic stress may induce the immune responses in mast cells. Treatment with fluoxetine can ameliorate pathological changes in gastric antrum of depressed rats, suggesting that SSRIs are an effective therapeutic agent for some gastroduodenal diseases caused by psychological factors.

**Terminology**
Rat mast cell protease-1 (MCP-1) is released and is associated with mast cell activation and degranulation.

**Peer review**
The authors of the present study showed that depression led to mast cell activation and degranulation in the gastric antrum. Fluoxetine counteracted such changes in gastric antrum in the depressed rat model.

**REFERENCES**

1. Stelekati E, Orinska Z, Bulfone-Paus S. Mast cells in allergy: innate instructors of adaptive responses. *Immunobiology* 2001; 212: 505-519
2. Yu LC, Perdue MH. Role of mast cells in intestinal mucosal function: studies in models of hypersensitivity and stress. *Immunol Res* 2001; 179: 61-73
3. Gui XY. Mast cells: a possible link between psychological stress and gastric mucosal integrity. *Immunol Rev* 2001; 179: 61-73
stress, enteric infection, food allergy and gut hypersensitivity in the irritable bowel syndrome. J Gastroenterol Hepatol 1998; 13: 980-989

4 Mayer EA, Craske M, Naliboff BD. Depression, anxiety, and the gastrointestinal system. J Clin Psychiatry 2001; 62 Suppl 8: 28-36; discussion 37

5 Kurina LM, Goldacre MJ, Yeates D, Gill LE. Depression and anxiety in people with inflammatory bowel disease. J Epidemiol Community Health 2001; 55: 716-720

6 Haug TT, Myklebust A, Dahl AA. Are anxiety and depression related to gastrointestinal symptoms in the general population? Scand J Gastroenterol 2002; 37: 294-298

7 Sykes MA, Blanchard EB, Lackner J, Koever L, Krasner S. Psychopathology in irritable bowel syndrome: support for a psychophysiological model. J Behav Med 2003; 26: 361-372

8 Possnerud I, Agerforz P, Ekman R, Bjørnsson ES, Abrahamsson H, Simren M. Altered visceral perceptual and neuroendocrine response in patients with irritable bowel syndrome during mental stress. Gut 2004; 53: 1102-1108

9 O’Brien SM, Scully P, Scott LV, Dinan TG. Cytokine profiles in bipolar affective disorder: focus on acutely ill patients. J Affect Disord 2006; 90: 263-267

10 Obuchowicz E, Marcinowska A, Herman ZS. [Antidepressants and cytokines–clinical and experimental studies] Psychiatry Pol 2005; 39: 921-936

11 Maes M. The immunoregulatory effects of antidepressants. Hum Psychopharmacol 2001; 16: 95-103

12 Kubera M, Lin AH, Genis G, Bosmans E, van Bockstaele D, Maes M. Anti-Inflammatory effects of antidepressants through suppression of the interferon-gamma/interleukin-10 production ratio. J Clin Psychopharmacol 2001; 21: 199-206

13 Wang GH, Dong HY, Dong WG, Wang XP, Luo HS, Yu JP. Protective effect of Radix Acanthopanacis Senticosi capsule on colon of rat depression model. World J Gastroenterol 2005; 11: 1373-1377

14 Yang PC, Jury J, Soderholm JD, Sherman PM, McKay DM, Perdue MH. Chronic psychological stress in rats induces intestinal sensitization to luminal antigens. Am J Pathol 2006; 168: 104-114; quiz 363

15 Dimitriadiou V, Lambrecht-Hall M, Reichler J, Theoharides TC. Histochemical and ultrastructural characteristics of rat brain perivascular mast cells stimulated with compound 48/80 and carbachol. Neurochem 1990; 39: 209-224

16 Letourneau R, Rozniecki JJ, Dimitriadiou V, Theoharides TC. Ultrastructural evidence of brain mast cell activation without degranulation in monkey experimental allergic encephalomyelitis. J Neuroimmunol 2003; 145: 18-26

17 Ide H, Itoh H, Tomita M, Murakumo Y, Kobayashi T, Maruyama H, Osada Y, Nawa Y. Cloning of the cDNA encoding a novel rat mast-cell proteinase, rMCP-3, and its expression in comparison with other rat mast-cell proteinases.

Biochem J 1995; 311 (Pt 2): 673-680

18 Barbara G, Stanghellini V, De Giorgio R, Corinaldesi R. Functional gastrointestinal disorders and mast cells: implications for therapy. Neurogastroenterol Motil 2006; 18: 6-17

19 Brown JK, Knight PA, Wright SH, Thornton EM, Miller HR. Constitutive secretion of the granule chymase mouse mast cell protease-1 and the chemokine, CCL2, by mucosal mast cell homologues. Clin Exp Allergy 2003; 33: 132-146

20 Theoharides TC, Spanos C, Pang X, Alferes L, Ligris K, Letourneau R, Rozniecki JJ, Webster E, Chrrousos GP. Stress-induced intracranial mast cell degranulation: a corticotropin-releasing hormone-mediated effect. Endocrinology 1995; 136: 5745-5750

21 Enck P, Frieling T. Neurogastroenterology–information processing from the viscera to the brain in humans. Dtsch Tierarztl Wochenschr 1998; 105: 468-471

22 Gally SJ. New insights into “the riddle of the mast cells”: microenviromental regulation of mast cell development and phenotypic heterogeneity. Lab Invest 1990; 62: 5-33

23 Csaba G, Kovacs P, Pallinger E. Hormones in the nucleus. Immunologically demonstrable biogenic amines (serotonin, histamine) in the nucleus of rat peritoneal mast cells. Life Sci 2006; 78: 1871-1877

24 Ferjan I, Erjavec F. Changes in histamine and serotonin secretion from rat peritoneal mast cells caused by antidepressants. Inflamm Res 1996; 45: 141-144

25 Purcell WM, Hanahoe TH. The activity of amitriptyline as a differential inhibitor of amine secretion from rat peritoneal mast cells: the contribution of amine uptake. Agents Actions 1990; 30: 41-43

26 Maes M, Kenis G, Kubera M, De Baets M, Steinbusch H, Bosmans E. The negative immunoregulatory effects of fluoxetine in relation to the cAMP-dependent PKA pathway. Int Immunopharmacol 2005; 5: 609-618

27 Wang Z, Hu SY, Lei DL, Song WX. [Effect of chronic stress on PKA and P-CREB expression in hippocampus of rats and the antagonism of antidepressors] Zhongguan Daxue Xuebao Yi Xueban 2006; 31: 767-771

28 Bondy B, Baghai TC, Minov C, Schule C, Schwarz MJ, Zwanzger P, Rupprecht R, Moller HJ. Substance P serum levels are increased in major depression: preliminary results. Biol Psychiatry 2003; 53: 538-542

29 Lieb K, Waiden J, Gruenze H, Feibich BL, Berger M, Normann C. Serum levels of substance P and response to antidepressant pharmacotherapy. Pharmacopsychiatry 2004; 37: 238-239

30 Budziszewska B, Jaworska-Feil L, Tetch M, Basta-Kaim A, Kubera M, Leskiewicz M, Lason W. Regulation of the human corticotropin-releasing-hormone gene promoter activity by antidepressant drugs in Neuro-2A and AT-T20 cells. Neuropsychopharmacology 2004; 29: 785-794

S- Editor Tian L  L- Editor Ma JY  E-Editor Ma WH