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Fluvoxamine inhibits some inflammatory genes expression in LPS/stimulated human endothelial cells, U937 macrophages, and carrageenan-induced paw edema in rat

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ABSTRACT

Objective(s): Fluvoxamine is a well-known selective serotonin reuptake inhibitor (SSRI); Despite its anti-inflammatory effect, little is known about the precise mechanisms involved. In our previous work, we found that IP administration of fluvoxamine produced a noticeable anti-inflammatory effect in carrageenan-induced paw edema in rats. In this study, we aimed to evaluate the effect of fluvoxamine on the expression of some inflammatory genes like intercellular adhesion molecule ($ICAM_1$), vascular cell adhesion molecule ($VCAM_1$), cyclooxygenases2 (COX_2), and inducible nitric oxide synthase (iNOS).

Materials and Methods: An *in vitro* model of LPS stimulated human endothelial cells and U937 macrophages were used. Cells were pretreated with various concentrations of fluvoxamine, from 10^{-8} M to 10^{-6} M. For *in vivo* model, fluvoxamine was administered IP at doses of 25 and 50 mg/kg^{-1}, before injection of carrageenan. At the end of experiment, the expression of mentioned genes were measured by quantitative real time (RT)-PCR in cells and in paw edema in rat.

Results: The expression of $ICAM_1$, $VCAM_1$, COX_2, and iNOS was significantly decreased by fluvoxamine in endothelial cells, macrophages, and in rat carrageenan-induced paw edema. Our finding also confirmed that IP injection of fluvoxamine inhibits carrageenan-induced inflammation in rat paw edema.

Conclusion: The results of present study provide further evidence for the anti-inflammatory effect of fluvoxamine. This effect appears to be mediated by down regulation of inflammatory genes. Further studies are needed to evaluate the complex cellular and molecular mechanisms of immunomodulatory effect of fluvoxamine.

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Introduction

Regarding the biological basis of depression, it has been proposed many years ago, that depression is a deficiency in monoamine neurotransmission. Later it was reported that there is a close connection between immune and nervous system and immune activation may be related to depression disorder. It has been demonstrated that inflammatory cytokine increase malfunctioning of noradrenergic and serotonergic neurotransmission (1). According to this hypothesis, antidepressant drugs could exert their therapeutic effect partially by attenuating the pro-inflammatory cytokine production or action, resulting in improvement of depressive symptoms (2). One of the crucial steps in the inflammation is adhesion and migration of leukocytes into the surrounding tissues. Binding and recruitment of circulating leukocytes to the vascular endothelium and migration into the subendothelial spaces, is the main process in the progress of inflammation. They are mediated through a cell adhesion molecules (CAMs) that up regulated on the vascular endothelium and leukocytes. Many studies illustrated that CAMs blockade can effectively inhibit inflammation. Among cell adhesion molecules, vascular cell adhesion molecule-1 (VCAM-1) and intracellular cell adhesion molecule-1 (ICAM-1) are well characterized. Evidence indicated that VCAM-1 and ICAM-1 play important roles in the firm attachment and transendothelial migration of leukocytes (3). Recent studies demonstrated that antidepressants are able to decrease migration of polymorphonuclear (PMN) leukocytes into the site of inflammation (4). So it is possible that

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antidepressants exert their effect on the migration of leukocytes through the influence on the expression of adhesion molecules, ICAM-1 and VCAM-1.

Other inflammatory mediators that up regulated in inflammation are COX2 and iNOS. COX2 is responsible for elevated levels of PGE2 and iNOS produces NO which leads to pathophysiological conditions (5–7). It seems that macrophages, both in the periphery and in the brain, are activated in depression and release pro-inflammatory cytokines that are responsible for activating the cyclooxygenase and nitric oxide synthase and increasing the inflammatory process (1).

SSRIs are broadly used in the treatment of major depression, panic disorder, obsessive–compulsive disorder and eating disorders. In comparison to other types of antidepressants, SSRIs have fewer side effects and better tolerated. Studies related to their anti-inflammatory activities are limited and to some extent are controversial (8).

Therefore in the present study we aimed to evaluate the effect of fluvoxamine one of SSRIs on the expression of ICAM-1, VCAM-1, iNOS and COX2 using an *in vitro* model system of LPS stimulated Human U937 macrophages and Human Umbilical Vein Endothelial cells (HUVECs) that has been a widely characterized model of the mammalian cellular response to various inflammatory stimuli and in carrageenan induced paw edema in rat model.

Materials and Methods

Chemicals

Human umbilical vein endothelial cells (HUVECs) and human monocytic cells (U937) were purchased from Pasteur Institute (Tehran, Iran). Dulbecco's minimal essential medium (DMEM), RPMI 1640 cell culture medium, fetal bovine serum (FBS), trypsin-EDTA and [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenylt-etrazolium bromide] (MTT) were obtained from Gibco. phorbol myristate acetate (PMA), lipopolysaccharide (LPS) from *Escherichia coli* 055:B5, dimethyl sulfoxide (DMSO), and dexamethasone were obtained from Sigma-Aldrich.

Fluvoxamine maleate was a gift from Abidi Pharmaceutical Co. (Tehran, Iran) and was dissolved in phosphate buffer saline (PBS) for cells and in isotonic saline for rats. Carrageenan (lambda) was purchased from Fluka Chemical (Switzerland) and was dissolved in isotonic saline.

Endothelial cell culture

HUVECs were cultured in T-75 flasks containing DMEM supplemented with 10% FBS. Antibiotics, penicillin (100 U/ml), and streptomycin (100 lg/ml) were added to the cell culture during the growth phase, but removed prior to experiment. At 70–80% confluence, cells were washed with PBS solution pH 7.4, and harvested with 0.025% trypsin – 0.01% EDTA. For experiments, cells were seeded in 6-well and 96-well plates and incubated for 24 hr. To choose the suitable concentration of LPS for endothelial cells stimulation, different amounts of LPS were diluted in PBS and added to the cells.

For the test, cells were treated with various concentrations of fluvoxamine from 10^{-8} M to 10^{-6} M. One hour later, the proper concentration of LPS (1 µg/ml) was added. Control cells and LPS (1 µg/ml) alone treated cells were also included. Control cells were incubated in DMEM alone (without LPS or component). Subsequently, cells were used for assessment of cell viability by MTT assay and for the measurement of mRNA levels of cell adhesion molecules ($VCAM_1$ and $ICAM_1$), COX_2, and iNOS by real-time PCR (RT-PCR).

Human U937 macrophages cell culture

The human monocyte cell line U937 was grown in complete RPMI 1640 medium supplemented with 10% (v/v) FBS, 100 units/ml penicillin, at 37 ˚C in a humidified atmosphere of 95% air and 5% CO_2. To differentiate the cells into adherent macrophages, they were seeded at a density of 5×10^5 cells/well in 12-well plates and incubated for 48 hr in the presence of PMA, at the final concentration of 100 nM. The cells were then washed and incubated in normal growth medium for additional 24 hr prior to the addition of LPS (1 µg/ml). Different concentrations of fluvoxamine from 10^{-8} M to 10^{-6} M were added to the medium, 1 hr before the addition of LPS (1 µg/ml). Cells with LPS alone and control cells (without LPS and test compound) were also included. Cells were used for the assessment of cell viability by MTT assay and for the measurement of mRNA levels of COX_2 and iNOS by RT-PCR.

Cell viability assay

To evaluate the toxicity of fluvoxamine and LPS at mentioned concentrations to endothelial cells and monocyte-derived macrophages, we used MTT assay. The ability of the cells to convert MTT shows mitochondrial activity and in consequence, cell viability (9). In this assay, cells were plated in 96-well plates at a concentration of 1×10^4 cells/well. Cells were incubated with different concentrations of LPS and various amounts of fluvoxamine from 10^{-8} M to 10^{-6} M, for 24 hr at 37 °C. After incubation, the medium was removed and replaced with 100 µl RPMI 1640 phenol red free. Then 10 µl of (12 mM) MTT stoke was added to each well. The cells were incubated for 4 hr at 37 °C. Finally the MTT crystals were dissolved by adding 50 µl of DMSO solution and the formazan blue dye was read in a microplate reader (BioTek Instruments, Epoch, USA) at 570 nm.

Animals

Male Wistar rats (200–250 g) were obtained from the animal house of the Faculty of Pharmacy, Isfahan University of Medical Sciences, Iran. Animals were housed in standard polypropylene cages, 4 per cage,

Table 1. Primers sequences of interested genes in human and rat

Homo sapiens		Rattus norvegicus	
Genes	Sequences (5′-3′)	Genes	Sequences (5′-3′)
$ICAM_1$	F: ACGGTGCTGGTGAGGAGAG R: TCGCTGGCAGGACAAAGGT	$ICAM_1$	F: CCACCATCACTGTGTATTCGTTCC R: ACGGAGCAGCACTACTGAGAG
$VCAM_1$	F: GCAAGTCTACATATCACCCAAG R: TCACAGAGCCACCTTCTT	$VCAM_1$	F: CTACATCCACACTGACGCTGAG R: CAGGGAATGAGTAGACCTCCACTT
COX_2	F: TGCAGTGAGCGTCAGGAG R: CAAGGATTTGCTGTATGGCTGAG	COX_2	F: ATTCTTTGCCCAGCACTTCACT R: CCTCTCCACCGATGACCTGATA
iNOS	F: GTCACCTACCACACCCGAGATG R: CGCTGGCATTCCGCACAA	iNOS	F: GAGAAGTCCAGCCGCACC R: CAATCCACAACTCGCTCCAAGA
18srRNA	F: TAGTCGCCGTGCCTACCA R: TGCTGCCTTCCTTGGATGT	18srRNA	F: GTTGGTTTTCGGAACTGAGGC R: GTCGGCATCGTTTATGGTCG

under a 12:12 hr light/dark cycle with free access to food and water. The experiments were carried out in accordance with local guidelines for the care of laboratory animals of the Isfahan University of Medical Sciences.

Carrageenan-induced paw edema and anti-inflammatory assay

Rats received a subplantar injection of 100 µl of a 1% (w/v) suspension of carrageenan lambda in the right hind paw (10). The volume of the paw was measured by Plethysmometer (Ugo Basile, Italy) immediately before and then 4 hr after the carrageenan injection. Data were expressed as the increase in paw volume (ml) and compared with pre-injection values.

In the present study 25 and 50 mg/kg doses were applied, according to our previous reports (11). This experiment contains five groups (n=6 rats in each group).

Fluvoxamine was injected IP, 30 min before subplantar injection of carrageenan. Control group received only vehicle. A group of animals that was pretreated with dexamethasone (1 mg/kg) was used as the positive control. Paw volumes (ml) were determined prior to carrageenan injection, and at the end of experiment (4 hr later) to determine the difference in paw volume. Then, animals were scarified and the inflamed paw tissues were cut. They were snapping frozen in liquid nitrogen and stored at −80 °C until they were used for RT-PCR.

Real-Time PCR

RT-PCR was performed for the detection of mRNA expression of $VCAM_1$, $ICAM_1$, COX_2, and iNOS. Total RNA was isolated from endothelial cells, U937 macrophages, and homogenized paw tissues by GeneJET RNA purification kit (Thermo Scientific, (EU) Lithuania), according to the manufacturer's instructions. The concentration and quality of RNA preparations were determined by a spectrophotometer (BioTek Instruments, Epoch, and USA) and gel electrophoresis. Standardized amounts of RNA were reverse-transcribed to cDNA using RevertAid first strand cDNA synthesis kit (Thermo Scientific, (EU) Lithuania) according to manufacturer's protocol.

The primers sequence for $VCAM_1$, $ICAM_1$, COX_2, and iNOS and housekeeping gene 18srRNA for human cell lines and rats were designed from the sequence list of GeneBank database (National Centre for biotechnology Information, NCBI) using Beacon designer 8 software, and then blasted against GeneBank database sequences. Primer sequences are presented in Table 1.

Real-Time PCR was performed using SYBRGreen (Thermo Scientific, (EU) Lithuania) detection in Corbett machine, Rotorgene 6000 (Australia). Master Mix in each reaction tube include, cDNA, H_2O, SYBR Green, forward and reverse primer of genes of interest.

The cycling conditions were as follows: initial denaturation at 95 °C for 3 min and 45 cycles of amplification (denaturation at 95 °C for 12 sec, annealing and extension at 60 °C for 45 sec). The relative amount of gene expression was normalized to the internal control 18srRNA. Validation of the reference gene (18srRNA) and the amplification efficiencies of targets and reference were performed (12). The fold-change for each sample was analyzed by the $2^{-\Delta\Delta CT}$ method. The $2^{-\Delta\Delta CT}$ values obtained from these analyses directly reflect the relative mRNA quantities for the specific gene in response to a particular treatment. Amplification of specific transcripts was further confirmed by obtaining melting curve profiles. All samples were run in duplicate.

Statistical analysis

The data are expressed as means±SEM. The differences between control and treatment groups were tested by one-way analyses of variance (ANOVA) followed by the Tukey *post hoc* test. Differences were considered as significant for $P<0.05$. Statistical analysis was performed using the SPSS 19 software.

Results

Cell viability

To investigate the cytotoxic effect of fluvoxamine and LPS on HUVECs and U937 macrophages, cells were treated with increasing concentrations of fluvoxamine (10^{-8} M to 10^{-6} M) and LPS (1 µg/ml) for 24 hr, and cell viability was measured using MTT assay.

These data from both endothelial cells and U937 macrophages showed that LPS alone and in addition of concentrations of fluvoxamine from 10^{-8} M to 10^{-6} M,

had no significant effect on cell viability compared to that of untreated cells (P<0.05) (Figure 1).

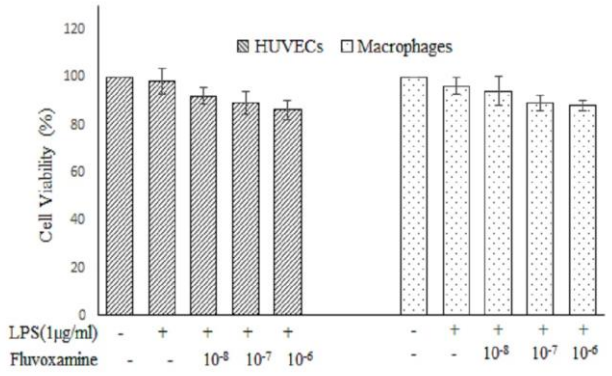

Figure 1. Effects of fluvoxamine and LPS on cell viability of HUVECS and U937 macrophages. Cells were treated with increasing amounts of fluvoxamine (10^{-8} M to 10^{-6} M) and LPS (1 µg/ml) for 24 hr. Values are expressed as Mean ± SEM of three independent experiments

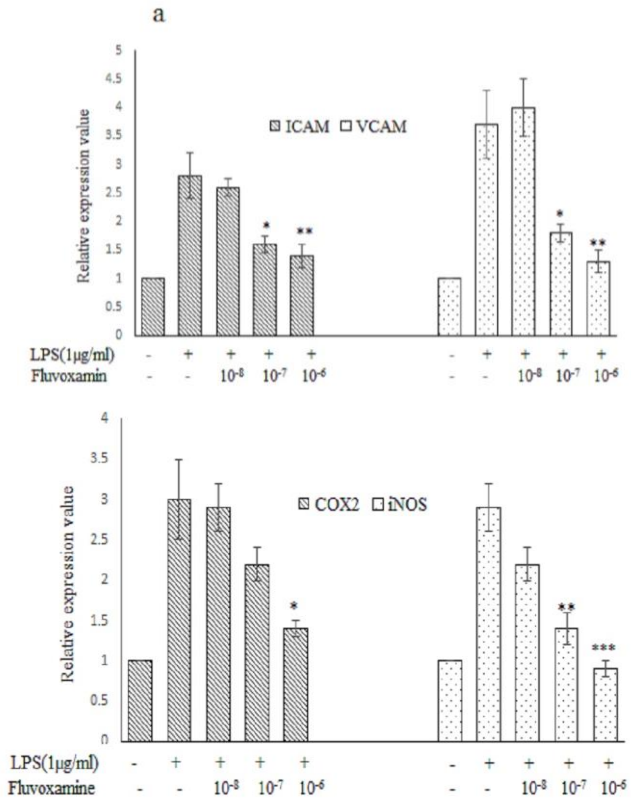

Figure 2. Effect of fluvoxamine on LPS-induced endothelial cells expression of $ICAM_1$, $VCAM_1$, COX_2, and iNOS. Cells were pretreated with the indicated amounts of fluvoxamine for 1 hr, and then activated with LPS (1 µg/ml). After 6 hr, RNA was purified from cells and transcription of $VCAM_1$ and $ICAM_1$ (a), and COX_2 and iNOS (b) was determined by quantitative RT-PCR. The mRNA expression data were normalized to the 18srRNA signal. Fold changes relative to control are presented as Mean±SEM. *P<0.05, **P<0.01, and *** P<0.001 compared with LPS alone treated group

Inhibition of LPS induced mRNA expression of $ICAM_1$ and $VCAM_1$ by fluvoxamine on HUVECs

To find the best concentration of LPS, we examined the effect of increasing concentrations of LPS on $ICAM_1$ and $VCAM_1$ genes expression in endothelial cells. LPS enhanced the expression of $ICAM_1$ and $VCAM_1$ genes in a concentration dependent manner (data not shown). 1 µg/ml of LPS with less toxicity significantly increased the $ICAM_1$ and $VCAM_1$ genes expression. As shown in Figure 2a, fluvoxamine meaningfully decreased the LPS-induced HUVECs expression of $VCAM_1$ at concentrations of 10^{-6} M (P-value = 0.006) and 10^{-7} M (P-value=0.01) compared with LPS alone treated group. Also, the expression of $ICAM_1$ was reduced significantly in the presence of fluvoxamine at 10^{-6} M (P-value<0.001) and 10^{-7} M (P-value=0.04) concentrations (Figure 2a).

Inhibition of LPS induced mRNA expression of COX_2 and iNOS by fluvoxamine on HUVECs

Fluvoxamine significantly suppressed the expression of iNOS gene at the concentrations of 10^{-6} M (P-value<0.001) and 10^{-7} M (P-value=0.001) in comparison to LPS alone treated group in endothelial cells. However, the expression of Cox_2 was reduced only at concentration of 10^{-6} M fluvoxamine (P-value=0.04) (Figure 2b).

Inhibition of LPS induced mRNA expression of COX_2 and iNOS by fluvoxamine on U937 macrophages

As shown in Figure 3, the expression of iNOS considerably decreased by fluvoxamine at 10^{-6} M and 10^{-7} M (P-value<0.001) concentrations. Fluvoxamine significantly decreased the COX_2 expression only at 10^{-6} M concentration (P<0.05). These data are compared with LPS alone treated group below (Figure 3).

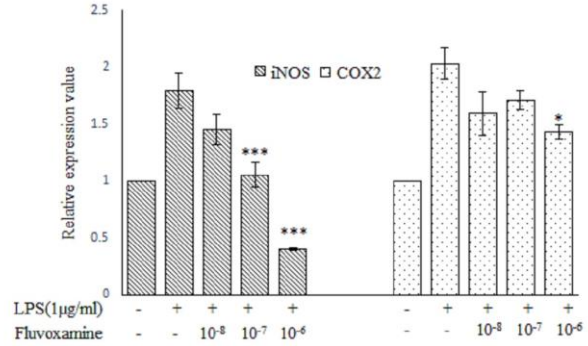

Figure 3. Effect of fluvoxamine on LPS-induced U937 macrophages expression of iNOS and COX_2. Cells were pretreated with the indicated amounts of fluvoxamine for 1 hr, and then activated with LPS (1 µg/ml). After 6 hr, RNA was purified from cells and transcription of COX_2 and iNOS was determined by quantitative RT-PCR. The mRNA expression data were normalized to the 18srRNA signal. Fold changes relative to control are presented as Mean±SEM. *P<0.05, *** P<0.001, compared with LPS alone treated group

Effect of IP injection of fluvoxamine on carrageenan induced paw edema

As demonstrated in Figure 4, IP administration of fluvoxamine at doses of 25 and 50 mg/kg considerably inhibited the development of paw edema as compared to the control group. As expected, the reference drug, dexamethasone (1 mg/kg), caused a significant inhibition of post-carrageenan edema (Figure 4).

The inhibitory effect of fluvoxamine on the expression of ICAM$_1$ and VCAM$_1$ in carrageenan induced paw edema in rats

To assess the *in vivo* anti-inflammatory effect of fluvoxamine, we measured the amount of $ICAM_1$ and $VCAM_1$ mRNAs in carrageenan-induced paw edema after the injection of fluvoxamine. As shown, carrageenan injection noticeably elevated the levels of these inflammatory genes. Remarkably, pretreatment with fluvoxamine significantly suppressed the gene expression in rat paw tissue.

Fluvoxamine similar to dexamethasone significantly decreased the expression of $VCAM_1$ at doses of 25 (P<0.001) and 50 mg/kg (P<0.001). Also, the expression of $ICAM_1$ was reduced significantly in the presence of fluvoxamine at doses of 25 (P<0.01) and 50 mg/kg (P=0.009) (Figure 5a).

The inhibitory effect of fluvoxamine on the expression of COX$_2$ and iNOS in carrageenan induced paw edema in rats

As illustrated in Figure 5b, fluvoxamine at doses of 25 and 50 mg/kg, considerably decreased the expression of iNOS (P=0.02, P=0.01) in carrageenan induced paw edema. COX-2 expression was significantly reduced by fluvoxamine at a dose of 50 mg/kg (P=0.04). As expected, the reference drug, dexamethasone (1 mg/kg), showed a significant iNOS and COX-2 mRNA reduction in carrageenan induced paw edema (Figure 5b).

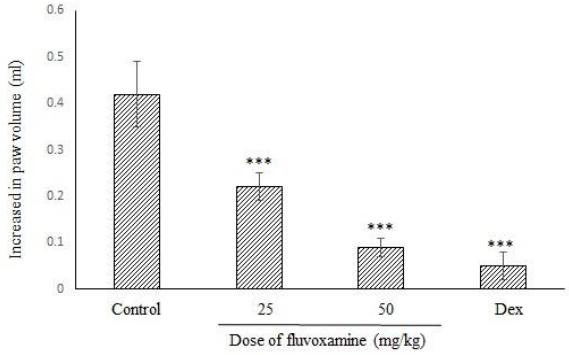

Figure 4. Effect of IP administration of fluvoxamine on carrageenan-induced paw edema in rat. Fluvoxamine or the vehicle was administrated 30 min prior to the carrageenan (1%) injection and rats were evaluated for paw edema at 4 hr post-carrageenan. A group of animals received dexamethasone (1 mg/kg) as a reference drug. Changes in the paw volume are presented as Mean±SD (n = 6, *** P<0.001 compared with control group). Dex: dexamethasone

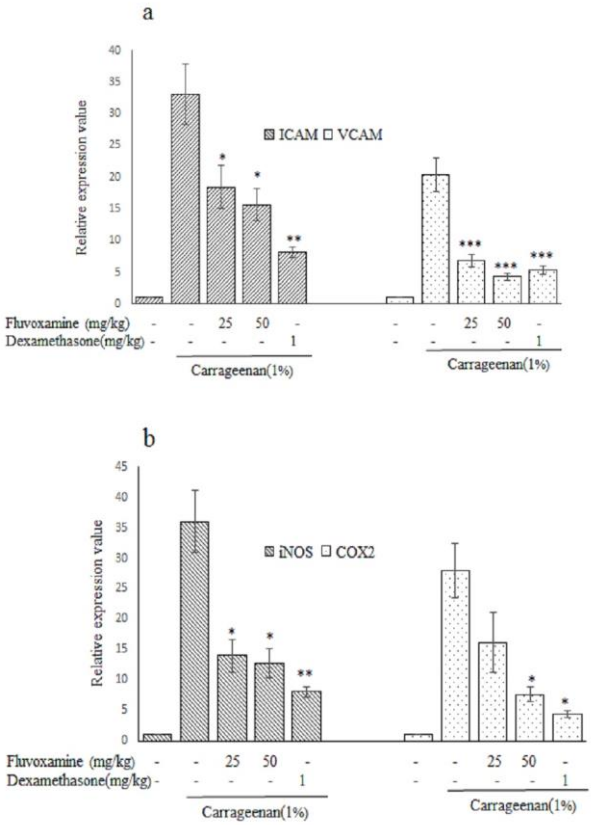

Figure 5. Effect of fluvoxamine on the gene expression of $ICAM_1$, $VCAM_1$ (a), iNOS, and COX_2 (B) in carrageenan-induced paw edema in rats. Fluvoxamine at doses of 25 and 50 mg/kg was given 30 min before subplantar injection of carrageenan (1%). Control group received only vehicle. A group of animals that was pretreated with dexamethasone (1 mg/kg) was used as the positive control. RNA was purified from inflamed frozen paw and transcription of $ICAM_1$, $VCAM_1$, iNOS, and COX_2 was determined by quantitative RT-PCR. The mRNA expression data were normalized to the 18srRNA signal. Fold changes relative to control are presented as Mean±SEM. *P<0.05, **P<0.01, and *** P<0.001 compared with only carrageenan received group.

Discussion

In the present study, fluvoxamine could affect the macrophages and endothelial cells to produce inflammatory markers, *in vitro*. We found that fluvoxamine could reduce the expression of $ICAM_1$ and $VCAM_1$ in LPS stimulated endothelial cells. Leukocytes attachment and migration into the intimal spaces, are mediated by adhesive molecules (VCAM and ICAM) expressed by activated endothelial cells (13, 14). Fluvoxamine inhibition of the expression of $ICAM_1$ and $VCAM_1$ suggests the anti-inflammatory effect of this antidepressant by inhibiting the migration of leukocytes to the inflammation site. This is confirmed by Lekakis and colleagues, that showed SSRIs (fluvoxamine, fluoxetine, and citalopram) decreased the TNF-alpha-induced endothelial expression of $VCAM_1$ and $ICAM_1$ (15). To confirm the anti-inflammatory effect of fluvoxamine we also measured the expression of COX_2 and iNOS in LPS-induced endothelial cells, two

known enzymes that elevate PGE_2 and NO levels in inflammation which are associated with many acute and chronic inflammatory diseases. We elucidated that fluvoxamine significantly decreases the expression of iNOS and COX_2 on mRNA levels in a concentration dependent manner.

A recent report showed that human peripheral blood mononuclear cells possess serotonin transporter, and it might be directly affected by antidepressants, especially by SSRIs (16– 19). Moreover, serotonin and noradrenaline released from lymphocytes and monocytes (20) can prompt immunomodulatory properties via receptors present on immune cells. Thus, we investigated the effect of fluvoxamine on the expression of iNOS and COX_2 in macrophages. It is well known that NO and PGE_2 are the main macrophage-derived inflammatory mediators. Anomalous production of NO and PGE_2 induces an inflammatory response that damages the adjacent cells and tissues of the host (6, 7). Thus, it has been assumed a good strategy to reduce inflammation through the suppression of these inflammatory mediators. Like endothelial cells, in macrophages, fluvoxamine inhibits the expression of iNOS and COX_2; however, the expression of COX_2 was reduced at higher concentration of fluvoxamine. Taler and colleagues investigated the effect of SSRIs on human T lymphocytes and showed the decreased expression of COX_2 with SSRIs (21). The concentrations we used for this *in vitro* study were close to therapeutic plasma concentrations (1 μM or below). Many *in vitro* studies used a higher concentration of antidepressant, so, the inhibitory effect was more pronounced (21, 22). We showed that inhibitory effect of fluvoxamine depends on concentration, and it is plausible that a higher concentration exerts more potent inhibitory effect on COX_2 expression. Several *in vitro* studies consistently have shown that antidepressants inhibit the NO production (23, 24).

As pointed out above, fluvoxamine inhibits the expression of $ICAM_1$, $VCAM_1$, COX_2, and iNOS in macrophages and endothelial cells. All 4 mentioned genes have a binding site in their promoter region for nuclear factor kappa B (NFκB), so, they might be induced by this transcription factor (6, 25–28).

Inflammation is a complex physiological process, and the anti-inflammatory effect of antidepressants cannot be explored just by *in vitro* study, so, we confirmed the anti-inflammatory activity of fluvoxamine in an *in vivo* carrageenan-induced paw edema model.

The findings of this study obviously showed that IP injection of fluvoxamine inhibits the development of paw edema over a period of 4 hr following carrageenan injection. These results are consistent with our previous reports that showed IP injection of fluvoxamine considerably inhibited paw edema response, 4 hr post-carrageenan injection (11). The expression of inflammatory mediators, $ICAM_1$, $VCAM_1$, COX-2, and iNOS was increased in inflamed rat paw tissue, and was inhibited significantly in fluvoxamine received groups in a dose dependent manner.

The anti-inflammatory effect of fluvoxamine especially at a dose of 50 mg/kg was similar to the anti-inflammatory effect of dexamethasone (1 mg/kg), a well-known glucocorticoid and potent immune-suppressive agent that may inhibit the expression of several cytokines and adhesion molecules (29). The acute carrageenan-induced inflammation is characterized by distinct phases, and a number of mediators are involved in the inflammatory response of carrageenan. The first phase occurs within 1 hr of carrageenan inflammation and is attributed to the release of histamine and serotonin. The second phase (over 1 hr) is mainly sustained by the migration of polymorphonuclear (PMN) cells to the inflammatory site which produce several pro-inflammatory mediators (30, 31). The present study demonstrated that fluvoxamine considerably decreases the expression of $ICAM_1$ and $VCAM_1$ in rat paw tissue, thus decreases the PMN leucocyte infiltration, and consequently inhibits the development of paw edema.

We showed that fluvoxamine inhibits the expression of COX_2 in rat paw edema. In agreement with our results, some studies have shown that tricyclic antidepressants and SSRIs attenuate PGE_2 synthesis (21). The expression of iNOS was also inhibited in paw tissue edema by fluvoxamine. As mentioned above, NFκB is a transcription factor involved in the increased expression of ICAM, VCAM, COX-2, and iNOS. In unstimulated cells, NFκB exists in the cytoplasm and binds to the inhibitory protein IκB. When the cells are exposed to the stimulants such as LPS or carrageenan, IκB is phosphorylated and releases NFκB, resulting in translocation of NFκB to the nucleus. Nuclear NFκB then binds to the promoters of pro-inflammatory mediators and induces the expression of target genes (32). Therefore, it is likely that transcriptional down-regulation of inflammatory genes by fluvoxamine is mediated by NFκB.

Furthermore, we assessed the viability of HUVECs and U937 macrophages in the presence of fluvoxamine; the examined concentration didn't change the cell viability. Previous study on human colon cancer cells and lymphocytes demonstrated that fluvoxamine modulates cell proliferation and reduces cell viability (33), however, low concentration of fluvoxamine applied in this study did not reduce the viability of HUVECs and U937 macrophages.

To our knowledge, this is the first study to evaluate the fluvoxamine anti-inflammatory effect on the expression of inflammatory mediators such as $ICAM_1$, $VCAM_1$, COX_2, and iNOS in an *in vitro* and *in vivo* model. Although, we investigated their expressions at mRNA level, their expression at protein level is suggested to be evaluated in future.

Conclusion

The results of the present study provide further evidence for the anti-inflammatory effect of fluvoxamine. Fluvoxamine potently inhibited the expression of $ICAM_1$, $VCAM_1$, COX_2, and iNOS genes at mRNA level, *in vivo* and *in vitro*. Further studies are needed to evaluate the complex cellular and molecular mechanisms of the immunomodulatory effect of fluvoxamine and its clinical relevance. Based on the potent anti-inflammatory effect of the drug, it may be a useful treatment in the management of inflammatory disease, especially in depressive patients who are suffering from inflammatory diseases. Modification of chemical structure of fluvoxamine might be promising for design and synthesis of new anti-inflammatory drugs with minimal SSRI side effects.

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